MONITORING ENERGY AND NITROGEN AVAILABILITY

FOR ARCTIC CARIBOU (*RANGIFER TARANDUS*)

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A

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ABSTRACT

Arctic caribou and reindeer (*Rangifer tarandus*) are an economically and ecologically important species. *Rangifer* populations are often affected by nutritional factors. Our ability to monitor nutrient supply to arctic ungulates is presently limited by a lack of techniques to consistently and easily measure availability of specific nutrients and which may disproportionately affect different segments of *Rangifer* populations. I refined and validated a method to measure availability of specific nutrients including nitrogen (N) and energy to caribou using purified fibrolytic enzymes and acid/pepsin to simulate digestion. I then used this method to measure how availability of nitrogen and energy was altered by anti-nutrients such as indigestible fiber and toxins. Digestible N contents in forages declined to almost zero by the end of the growing season, whereas digestible energy concentrations were still sufficient to meet basic maintenance requirements for caribou by the end of the growing season in shrub and forb forages. Shrubs contained the highest amounts of total N and energy, however this was reduced by fiber and toxins so that shrubs contained the lowest digestible N contents, especially for *Betula nana*. Graminoids were extremely low in digestible energy content, which may necessitate a high degree of selection among plant parts by herbivores.

Dietary choice over long- and short-term periods may be assessed using non-invasive stable isotope techniques, nevertheless, the understanding of how isotopic signatures vary over spatial, temporal, and species-specific scales and how isotopic signatures are changed by digestive processes is limited. Monocot (graminoid) and dicot (browse and forb) forages both differed in values of 13C and 15N, however regional and seasonal shifts in 13C were larger than the differences among forage groups themselves. Forage isotopic signatures also changed after

simulated digestive processes, yet this was only significant for species with very low (< 52.6 % N) or very high (> 36.6 % C) digestibilities.

These studies suggest that nitrogen may be a limiting nutrient for caribou populations. Persistence of arctic caribou populations in a changing climate may depend, in part, upon continued access to calving grounds, the change in abundance of individual shrub species, and/or the ability of caribou to behaviorally and physiologically cope with increasing amounts of toxins in shrubs.

DEDICATION

This thesis is dedicated to my grandmother,

Eleanor Seiter Douglass

May 8, 1923 – July 16, 2013

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CHAPTER 1: INTRODUCTION

Importance of Herbivores to Arctic Ecosystems and Economies

Arctic herbivores play a myriad of roles that are vital to northern ecosystems by depositing N and other minerals used by plants (Hik and Jefferies 1990), shaping the underground landscape (Wheeler and Hik 2013), changing plant biomass (Bråthen and Oksanen 2001), and altering species composition (Augustine and McNaughton 1998). Wild and domesticated arctic ruminants such as moose (*Alces alces*), caribou (*Rangifer tarandus granti, R. t. caribou*, and *R. t. pearyi)*, reindeer (*R. t. tarandus*), and muskoxen (*Ovibos moschatus*) are also utilized for meat, milk, and wool and contribute significant amounts of money to economies in Alaska (EcoNorthwest et al. 2014, McDowell McDowell Group 2014), Scandinavia (Nieminen and Kemppainen 1999, Storaas et al. 2001), and other parts of the circumpolar north (Hudson 1989, Humphries 2007). Arctic herbivores form such a critical component of northern human-environmental interactions that they are often an inseparable part of indigenous culture (King 2002, Hummel and Ray 2008).

Influence of Nutrient Types on Herbivore Populations

Nutrition may be one of the dominant factors limiting population growth of arctic herbivores. Population fluctuations of many arctic caribou herds have been linked to climatic (Grayson and Delpech 2005, Tyler 2010, Joly et al. 2011) and demographic (Messier et al. 1988, Tews et al. 2007) patterns that affect access to and quality of forage, and hence alter nutritional status and mortality of animals. Climatic and demographic influences on nutrition have also been implicated in regulating population sizes of rodents (Batzli 1983), geese (Morrissette et al. 2010), and a wide range of other arctic herbivores (White 2008). Furthermore, predator densities in the arctic are generally low. This is manifested in typically high offspring survival rates of many

arctic herbivores, especially in migratory populations who give birth and remain in areas of low predator concentrations for extended periods of time (Whitten et al. 1992).

Demographic parameters such as recruitment and survival are influenced by different categories of nutrients. Nutrients can be broadly classified into two classes – micronutrients such as minerals and vitamins and macronutrients including protein, lipids, and carbohydrates that are the basis for energy flow (Barboza et al. 2009). Micronutrients are important for many herbivore populations (e.g., Barboza et al. 2003), however macronutrients, by definition, are required in larger quantities and may therefore be the most important limiting factor for arctic herbivore populations. Nutrient content may affect different demographic parameters disproportionately because different nutrients are routed to specific physiological purposes within the bodies of herbivores (Allaye-Chan 1993).

Recruitment of new individuals into the population is directly influenced by protein status of reproductive females. In ungulates, demands for nitrogen (N), a common proxy for protein, are high during pregnancy and lactation, and can even exceed the N demands of the mother herself (Barboza and Parker 2008, Barboza et al. 2009, Hackmann 2011). Large N demands occur during fetal development throughout the winter (Barboza and Parker 2008), during periods when N intake is often at an annual minimum. Reproductive females without sufficient stores of N may abort fetuses (Russell et al. 1998), give birth to an underdeveloped calf (Roffe 1993), or reduce the allocation of body N to their milk (Taillon et al. 2013). Reproductive females can draw upon N stores in muscle and organ tissue throughout much of the year when N demand exceeds intake (Gerhart et al. 1996, Barboza and Parker 2006) and thus herbivores have acquired numerous mechanisms to conserve protein and the function of their muscles and organs (Parker

et al. 2005). Despite this fact, N is often the single greatest limiting factor for recruitment in many herbivores populations (White 1993, DeGabriel et al. 2009, McArt et al. 2009).

Survival of adults and calves is affected by their ability to meet basic maintenance requirements for energy expenditure. Herbivores can catabolize all three macronutrient types – protein, lipid, and carbohydrates – to fuel energy expenditures. Plant structural and storage carbohydrates comprise the largest fraction of herbivore diets that can be converted into fats and stored in localized fat deposits by the animal during seasonal periods of mass gain. Large amounts of fat deposits provide a consistent energy source throughout the winter and minimize catalysis of more limited protein stores in organs and muscle tissue (Parker et al. 2005). Thus, fat stores secondarily affect reproduction by sparing maternal protein stores from catalysis so that they may be preferentially deposited into offspring (Barboza and Parker 2008). Fat stores can also affect reproduction during lactation, when energy is transferred to the offspring through milk (Hackmann 2011). There are many correlative observations between size of fat deposits in reproductive females and reproductive success (Thomas 1982, Crête and Huot 1993) because energy reserves influence survival (Cuyler and Øritsland 1993, Cook et al. 2004) as well as maternal investment during pregnancy and lactation (Barboza and Parker 2008).

Nutrient Acquisition

The ability of herbivores to obtain nutrients is partially determined by the nutrient content of forages and further modified by the digestibility of those nutrients. Nutrient concentrations in arctic forages are highly seasonal and, for many nutrient types, insufficient to meet reproductive and maintenance demands throughout large periods of the year (Gerhart et al. 1996, Ohlson and Staaland 2001). Thus, great emphasis has been placed on assessing range quality for herbivores by measuring total nutrient concentrations (usually of N and minerals) in forages throughout the

growing season. Declines in nutrient concentrations over the course of the season are also generally accompanied by declines in nutrient digestibility, as indigestible structural fibers make up an increasing proportion of forage plant tissues (Van Soest 1984, Chapin et al. 1986). Although measurement of total nutrient concentrations are generally simple and fast, digestibility of nutrients are often only assessed as indices and rarely measured directly (e.g., dry matter [DM] digestibility; Person et al. 1980, Côté 1998, Storeheier et al. 2002a).

Nutrient content and digestibility also vary between forage types (e.g., forbs, shrubs, and graminoids), species within forage types, and even individual plant parts. Consequently, herbivore diets often shift substantially throughout the course of the growing season, as different forage species change in quality and quantity. For example, seasonal diets of the Porcupine caribou herd shift from a graminoid-dominated diet in early summer to a shrub-dominated diet in mid to late summer (Thompson and McCourt 1981, Russell et al. 1993) as these forages become available at their peak nutritional content at different times during the spring (Johnstone et al. 2002). Among forage types, graminoids can occur at very high densities in certain habitat types (e.g. wet sedge meadows in early June, where graminoids represent 86% of total aboveground biomass; Russell et al. 1993) but are often considered lower-quality food due to the high fiber content (60 - 80% NDF; Johnstone et al. 2002) and low N content (1 - 3% N; Johnstone et al. 2002) within leaves compared to browses, which is often found at lower densities than graminoids, especially early in the growing season. Browse forages are generally regarded as the highest-quality food due to their low fiber content (20 - 30% NDF; Johnstone et al. 2002) and high N content (1 - 5% N; Johnstone et al. 2002). There is some uncertainty into the absolute amount of digestible nutrients within browse species, however, because nutrients are often made unavailable to the animal by high concentrations of plant defense compounds such as plant

secondary metabolites (PSMs; Robbins et al. 1987, Hanley et al. 1992, McArt et al. 2009). Herbivores may be able to use chemical or behavioral means to limit negative effects of PSMs, however recent work suggests that browsing caribou may not necessarily be able to avoid high intake of PSMs when feeding exclusively on browse (Thompson and Barboza 2013). Among mixed browser-grazers, variation in the selection of forage species and plant parts can affect nutrient intakes that ultimately alter nutritional condition and thus reproduction (White 1983).

Measurement of Digestibility

Methods for measurement of forage digestibility vary widely in complexity, infrastructure and cost. Animal-based methods are popular but require either access to captive herds of surgically altered animals (e.g., Person et al. 1980, Côté 1998) or harvesting of digestive fluids from freshly killed animals (e.g., Storeheier et al. 2002a, Storeheier et al. 2002b), preferably of the same species of interest. These methods are, at best, expensive and difficult to execute, and may not even be feasible for some herbivores such as endangered species and/or animals not amenable to captive situations. Thus, many researchers have turned to methods based on commercially available purified enzymes, which are much cheaper and easier to use (Tamminga and Williams 1998), despite the fact that these techniques may not be as accurate as animal-based methods in measuring true digestibility. Methods involving incubation within fistulated animals and/or laboratory incubators, with and/or without commercial purified enzymes, and with varying lengths of incubation have all been applied without agreement on a common standard (Kitessa et al. 1999, López 2005).

Digestibility measured using either animal-based methods or purified enzymes also involve trade-offs between accuracy and precision. Animal-based methods capture more of the variation associated with digestive physiology, but large amounts of individual variation between

animals and periods of time limit the reproducibility of this approach and often increase the variation around the estimates of digestibility (Kitessa et al. 1999, López 2005, Mohamed and Chaudhry 2008). Thus, digestibility estimates derived from these animal-based techniques are difficult to compare across different regions and time periods. Although purified enzymes do not fully capture physiological processes associated with digestion, they are more consistent and more readily reproduced for comparisons between laboratories and samples (Kitessa et al. 1999, López 2005). Digestibility estimates obtained using purified enzymes can also be linked to animal-based estimates with relationships derived from direct measures of digestibility in animals.

A major advantage of purified enzyme methods is the ability to measure the digestibility of specific nutrients. Traditionally, measurement of digestibility has been limited only to DM, with the assumption that this is only a measure of relative forage quality or that individual nutrients (e.g., energy, protein, and minerals) are digested at the same rate as DM. For example, Peltier et al. (2003) reported the DM digestibility of grass hay in muskoxen during the spring was 69%, whereas the digestibility of N, S, and Ca was only 61%, 30%, and 28%, respectively. Animal-based methods are unable to measure digestibilities of individual nutrients without specific markers because the microbes and endogenous secretions responsible for digesting the sample also contaminate the residues and are unable to be removed by simple washing (Ihl and Barboza 2007). Purified enzymes are easily washed out of samples, however, thus leaving contamination-free residues which can be easily assessed for digestibility of individual nutrients.

Measurement of Diet Composition

Diet composition of wild animals has often been measured from partially digested residues of food in the digestive tract or the feces. These techniques rely on identifying and

quantifying fragments of indigestible plant materials, which biases the samples because highly digestible items may not even be present in digesta or feces (Boertje 1981). Digestibility corrections are applied to correct for detection bias (e.g., Boertje 1981), however these corrections assume that digestibility remains constant – an assumption which is likely to be violated by changing and variable digestibilities in wild forages (Gill et al. 1983, Mayes and Dove 2000). Additionally, because these techniques rely on material that has recently been eaten, it is only possible to determine dietary composition over a relatively short period of time which may not match long-term consumption patterns, especially for mixed feeders. For example, diets of the Porcupine caribou herd are dominated by lichens, graminoids, and willows at various times of the year (Thompson and McCourt 1981).

Many ecologists are now using stable isotopes to infer diet composition because these techniques can integrate dietary information from differing time periods depending on the types of tissues sampled (Gannes et al. 1998, Crawford et al. 2008). Nevertheless, there is a large potential for error when estimating diet composition with stable isotopes (Gannes et al. 1997, Boecklen et al. 2011). For example, small differences in isotopic composition among diet items make it difficult or impossible to distinguish individual diet sources (Crawford et al. 2008). Dietary compositions can also be inaccurate if isotopic signature of diet items changes over time or in different locations and are not accounted for in mixing models (Crawford et al. 2008). The largest factor affecting accurate diet composition estimates, however, may be the fractionation value – i.e., the systematic difference in isotopic composition between the diet source and the animal tissue (Caut et al. 2009, Martinez del Rio et al. 2009).

Dietary reconstructions of herbivores using stable isotopes are further hampered in arctic ecosystems because large differences in the traditional herbivore "diet" isotope, ^{13}C, do not exist

in this ecosystem (Mayes and Dove 2000). In tropical and temperate ecosystems, forage types are clearly separated by differences in isotopic ratios of C ($\delta^{13}C$) values of 12-14‰ (Crawford et al. 2008) between high-quality C_3 browse species and low-quality C_4 graminoid species. However, C_4 plants are absent from arctic systems (Öpik and Rolfe 2005), and thus only small differences in $\delta^{13}C$ values are available for dietary reconstructions of herbivores. Instead, stable isotope ratios of N ($\delta^{15}N$) may be used to determine herbivore diet in arctic ecosystems, however patterns in distribution and animal-tissue fractionation are less common for this isotope due to the preponderance of attention on ^{13}C as a dietary tracer in tropical and temperate ecosystems (Mayes and Dove 2000).

Fractionation values are also often applied across studies with little research on the mechanisms behind fractionation (Caut et al. 2009). Several authors have noted that fractionation values seem to be affected by forage quality. In particular, Florin et al. (2011) noted that fractionation values of $\delta^{15}N$ were heavily influenced by the quality of dietary proteins (as defined by the relative content of the most limiting amino acid). Furthermore, Codron et al. (2011) noted that $\delta^{13}C$ values of high-quality C_3 plants were incorporated at much faster rates and, upon equilibration, were in fact overrepresented within the tissues of animals. Fractionation values may be further skewed by PSMs within forages because these anti-nutrients alter the digestibility of nutrients (and presumably the isotopes composing these nutrients), yet this idea has not been tested before.

Objectives

My objectives were to 1) evaluate a new method to determine forage quality for arctic herbivores, 2) assess the quality of the summer range of the Central Arctic caribou herd, and 3) assess and identify sources of variation in common stable isotope values which could affect their

use for diet estimation of herbivores in arctic ecosystems. My research questions were designed to address the following questions:

1. How do fiber and phenolics limit N availability to caribou?
2. Is digestible N sufficient for maintenance and reproduction in Central Arctic caribou on their summer range?
3. What sources of variation could affect the use of stable isotopes to estimate diet in caribou?

In **Chapter 2**, I refined a new method to measure digestibility of DM and of specific nutrients. I validated this method using estimates of DM digestibility obtained from incubation within three fistulated, non-reproductive female reindeer.

In **Chapter 3**, I collected vascular forage samples from the summer range of the Central Arctic caribou herd across a temporal and latitudinal gradient. I analyzed these samples for concentrations of nutrients and anti-nutrients, and examined how these two factors interacted to affect concentrations of digestible energy and N. I also analyzed forages for variations in stable isotope ratios of C and N among species, location, and time. I then calculated fractionation values between the whole plant and corresponding indigestible residue and digested fraction to examine how fiber and phenolics affected fractionation of these two components.

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CHAPTER 2: MONITORING DIGESTIBILITY OF FORAGES FOR HERBIVORES: A NEW APPLICATION FOR AN OLD APPROACH[1]

Abstract

Ruminant populations are often limited by how well individuals are able to acquire nutrients for growth, maintenance, and reproduction. Nutrient supply to the animal is dictated by the concentration of nutrients in feeds and the efficiency of digesting those nutrients (i.e., digestibility). Many different methods have been used to measure digestibility of forages for wild herbivores, all of which rely on collecting rumen fluid from animals or incubation within animals. Animal-based methods can provide useful estimates but the approach is limited by the expense of fistulated animals, wide variation in digestibility among animals, and contamination from endogenous and microbial sources that impairs the estimation of nutrient digestibility. We tested an *in vitro* method using a two-stage procedure using purified enzymes. The first stage, a six hour acid-pepsin treatment, was followed by a combined seventy-two hour amylase-cellulase or amylase-Viscozyme treatment. We then validated our estimates using *in sacco* and *in vivo* methods to digest samples of the same forages. *In vitro* estimates of dry matter (DM) digestibility were correlated with estimates of *in sacco* and *in vivo* DM digestibility (both $P < 0.01$). The *in vitro* procedure using Viscozyme ($r^2 = 0.77$) was more precise than the *in vitro* procedure using cellulase ($r^2 = 0.59$). Both procedures can be used to predict *in sacco* digestibility after correcting for the biases of each method.

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We used the *in vitro* method to measure digestibility of nitrogen (N; 0.07 – 0.95 g/g), which declined to zero as total N content declined below 0.03 – 0.06 g/g of DM. The *in vitro* method is well suited to monitoring forage quality over multiple years because it is reproducible, can be used with minimal investment by other laboratories without animal facilities, and can measure digestibility of individual nutrients such as N.

Introduction

The amount of feed that an animal must consume to meet its demands for maintenance and reproduction is influenced by the efficiency of digestion, which can be expressed as the digestibility: the proportion of dry mass (DM) or nutrients an animal is able to absorb from the diet. Digestible energy and nitrogen (N) may directly limit population size by limiting both survival and reproduction of a wide variety of herbivorous mammals from marsupial possums (*Trichosurus vulpecula* (Kerr, 1792)) to moose (*Alces alces* (L., 1758)) and colobine monkeys (DeGabriel et al. 2009; McArt et al. 2009; Wallis et al. 2012). Large herbivores that graze in extensive rangelands are often faced with wide variations in both the nutrient content and digestibility of feeds (Klein 1981, 1990; Russell et al. 1993; Johnstone et al. 2002; Mårell et al. 2006; Finstad and Kielland 2011). In addition, temperate and northern herbivores such as migratory caribou (*Rangifer tarandus granti* (J.A. Allen, 1902) and *R. t. groenlandicus* (L., 1767); Festa-Bianchet et al. (2011)) and reindeer (*R. t. tarandus* (L., 1758)) are constrained by a short period of plant growth in which their nutritive value and digestibility vary with season (Klein 1990).

Herbivores typically consume feeds with less crude protein and more fiber than those consumed by omnivores or carnivores. Vertebrate herbivores depend on microbial fermentation

to digest the complex carbohydrates in plant fiber (Dehority 2003; Sundset et al. 2004; Barboza et al. 2009). Several methods are commonly used to determine digestibility in wild herbivores, all of which rely on fermentative digestion in animals at some stage. *In sacco* digestibility methods consist of incubation of a forage sample for fermentation within the rumen of a fistulated animal (Kitessa et al. 1999; Mohamed and Chaudhry 2008). After incubation, samples are often (but not always) further degraded with an *in vitro* acid-pepsin treatment to simulate passage through the gastric region or abomasum (Tilley and Terry 1963). Other *in vitro* techniques rely on collection of rumen fluid from fistulated or freshly-killed animals for sources of microbes to digest forages (Tamminga and Williams 1998). Forage samples are then incubated with rumen fluid in laboratory settings rather than inside the animal. *In vivo* techniques measure digestion by mass balance or with indigestible markers to estimate the fraction of a diet that is lost when feed passes through the animal (Barboza et al. 2009).

An ideal method for monitoring digestibility would be simple, cheap, repeatable in time and across laboratories, and able to analyze many samples quickly. Animal-based digestibility methods are insufficient for monitoring forage quality because they are difficult to replicate (Ayres 1991). Digestion in animals is not a static process, and can vary with species, season, physiological status, feed intake, and interactions among feeds consumed (Thomas et al. 1984; Ayres 1991; Kitessa et al. 1999; Mohamed and Chaudhry 2008; Niderkorn and Baumont 2009). Furthermore, animal-based methods are often unavailable to wildlife researchers because these methods rely on captive populations that are expensive to maintain and subject to extensive regulation for animal use and welfare (Mohamed and Chaudhry 2008). Estimates of nutrient digestibility from animal-based methods are also confounded by contamination of the forage from microbial and endogenous sources (Krawielitzki et al. 1999; Marini et al. 2008).

In vitro methods of measuring digestibility with purified enzymes have been applied to feeds for domestic animals (Boisen and Eggum 1991; Tamminga and Williams 1998) but have only been used occasionally on feeds for wild herbivores (DeGabriel et al. 2009). In comparison with animal-based methods, *in vitro* methods are more appropriate for monitoring digestibility because they are more consistent, require less infrastructure, are less costly, and can measure digestion of specific nutrients without the confounding effects of microbial and endogenous materials (Kitessa et al. 1999; DeGabriel et al. 2008). *In vitro* methods may underestimate DM digestibility because they use purified enzymes that may be less efficient than those produced by microbes and the tissues of the animal (Kitessa et al. 1999; López 2005). Purified enzymes may be subject to end-product inhibition (Van Soest 1984; Tamminga and Williams 1998) because enzymes and substrates are not continually being replaced under *in vitro* conditions. Furthermore, purified enzymes may be more vulnerable than endogenous enzymes to interference and inhibition by plant secondary metabolites (PSMs; Van Soest 1984). Nevertheless, *in vitro* methods can provide accurate estimates of digestibility in the animal when validated against animal-based approaches (Boisen and Eggum 1991).

Our primary objective was to develop an *in vitro* method to approximate the true digestibility of a wide range of reindeer feeds as closely as possible. To achieve this primary objective, we had several secondary objectives. Our first secondary objective was to achieve optimal incubation conditions for *in vitro* digestion. We adapted techniques from Tilley and Terry (1963), Choo et al. (1981), and DeGabriel et al. (2008), and also tested new incubation materials and methods to achieve this goal. Our next secondary objective was to determine physical and chemical properties of samples and digestion materials that would affect *in vitro* and *in sacco* digestion estimates, because it is common for researchers to mix and match

components of different techniques. Our next secondary objective was to evaluate whether *in sacco* estimates are reasonable estimates of whole-gut (*in vivo*) digestion in reindeer, because it is not possible to measure a wide range of samples using the *in vivo* approach. Finally, our last objective was to determine the digestible nutrient (N and C) content of animal feeds in reindeer. We used semi-domesticated reindeer for our study because, similar to migratory caribou in North America, this subspecies experiences wide seasonal variation in forage quality throughout its annual cycle.

Materials and Methods

We used a standard reference set of feed samples for digestion measurements (Table 1). The reference set was comprised of mixed diets available to captive reindeer, agricultural feed components commonly used for captive reindeer, and wild forages that were naturally available to caribou. Samples of mixed diets and their ingredients were kept cool until they could be stored at -20° C, which was usually within 2 hours of collection. Stored samples were thawed and oven-dried to constant mass in a convection oven at 50° C. Samples of forages were air-dried in the field and oven-dried to constant mass in a convection oven at 50° C. All dried samples were ground through #20 mesh (1.27 mm) in a Wiley mill (Arthur Thompson Co., Philadelphia, PA).

Samples of forages are frequently preserved using both freeze-drying and oven-drying techniques, so we tested the effect of drying technique on *in vitro* dry matter (DM) and N digestibility. We collected fresh willow leaves (24 samples of *Salix pulchra* (Cham.) and 9 samples of *S. richardsonii* (Hook.)) along the northern portion of the Dalton Highway in the range of the Central Arctic Caribou herd in Alaska (Arthur and Del Vecchio 2009). We stored a subsample (approximately 70 g) of leaves at -20°C while the remainder was air-dried in the field. Frozen samples were freeze-dried in a lyophilizer (Labconco Model 7755044, Kansas City, MO)

to constant mass whereas air-dried samples were oven-dried to constant mass in a convection oven at 50º C. All willow samples were ground as described above.

We established a protocol for *in vitro* digestion after measuring DM loss from incubations with buffers and enzymes over several time steps. We then analyzed how physical and chemical properties of the forages and the incubation bags affected estimates of *in vitro* DM digestibility. Our *in vitro* estimates were compared with those from foregut digestion (*in sacco*) to provide predictive relations between *in vitro* and *in sacco* DM digestibility. We compared measures of DM digestibility over the whole digestive tract (*in vivo*) with those of only the foregut (*in sacco*) and with those measured *in vitro* in order to verify that measures of foregut digestibility are valid proxies of whole gut digestibility in reindeer. Finally, we quantified the digestibility of N and carbon (C). We then compared the N and C digestibilities to DM digestibility, and concentrations of digestible N to total N of forages.

Method Development – In vitro Assay

Forage samples were incubated in polyester bags (#F57 fiber filter bags at 25 µm pore size; Ankom Technology, Macedon, NY) that were also used for detergent extraction of fiber. To remove any materials that would inhibit microbial or enzyme activity (Ankom Technology 2005), filter bags were rinsed in acetone twice for 3 minutes, oven-dried for 24 hours at 50º C, and weighed to the nearest 0.0001 g. We then loaded 0.5 g of sample into a weighed bag, the bag was heat sealed, and oven-dried for 24 hours at 50º C. We used empty bags as controls and included an alfalfa (*Medicago sativa* (L.)) standard for every group of 24 samples. Bags were rinsed four times in distilled water and dried for 48 hours at 50º C after treatment with enzyme solutions. All samples were assayed in triplicate.

We used three solutions to simulate digestion in the foregut: two solutions to digest structural carbohydrates (cellulase or Viscozyme) and one solution for acid digestion. We used a pH 5.0 buffer composed of 37% v/v 0.1 M glacial acetic acid and 63% v/v 0.1 M anhydrous sodium acetate for carbohydrate digestion because this was the optimal pH for the carbohydrase enzymes we tested. We used α-amylase (2 mL/L; Ankom Technology, Macedon, NY) with either cellulase (1.8mL/L at 110.2 units/mL anhydrous sodium acetate/glacial acetic acid buffer solution; Sigma-Aldrich, St. Louis, MO; Catalog No. C0615), or a fibrolytic enzyme mixture (8 mL/L of Viscozyme; Sigma-Aldrich, St. Louis, MO; Catalog No. V2010). The cellulase solutions were first used to establish the protocol. We subsequently used Viscozyme solutions to test if DM digestibility was improved by inclusion of a wider range of carbohydrases. Acid-pepsin digestion occurred at pH 1 in a 0.1 N HCl solution that contained 2 g/L pepsin (1:10,000 [10,000 IU/mg]; MP Biomedicals, Solon, OH; Catalog No. 102598; Tilley and Terry 1963).

To verify that enzymes enhanced DM digestibility beyond simple dissolution of the sample, we used a mixed diet (high fiber/ high protein ration) to test amylase and cellulase activity over 48 hours in three different solutions: a control buffer solution, a buffer solution with only amylase, and a buffer solution with only cellulase. Similarly, we tested pepsin activity on the same mixed diet over 48 hours in two different solutions: a control acid solution, and an acid solution with pepsin. We determined optimal incubation times for digestion of three forages with different concentrations of fiber and protein (alfalfa, corn kernels (*Zea mays* (L.)), and straw (*Triticum* sp. x. *Secale* sp.); Table 1). Acid-pepsin digestion was tested at 6, 24, and 48 hours of incubation. Amylase-cellulase digestion was tested at 24, 48, or 72 hours of incubation after first digesting samples for 6 hours in acid-pepsin. Finally, we determined the optimal order of incubation steps: we digested samples of the same mixed diet and alfalfa in amylase-cellulase

both before and after acid-pepsin digestion. All steps of the *in vitro* assay were performed with 1 L of digestion solution for each group of 24 samples. Samples were incubated in rotating glass jars within a Daisy *In vitro* Incubator (Ankom Technology, Macedon, NY) at 37ºC with constant rotation.

We established a final protocol of incubation times, which was used to test the effect of pore size on estimates of *in vitro* DM digestibility. We used a small subset of samples representing a range of fiber concentrations (alfalfa, corn kernels, and straw; Table 1) to compare *in vitro* digestibility measures between bags with 25 µm and 50 µm pore sizes (F57 bags [4.5 x 5 cm] vs. 5 x 10 cm concentrate bags; Ankom Technology; Macedon, NY).

In sacco and In vivo DM Digestibility

All reindeer used in these experiments were held at the Robert G. White Large Animal Research Station in Fairbanks, Alaska and handled according to procedures approved by the Institutional Animal Care and Use Committee under protocol # 131442. Reindeer were provided with fresh water and feed *ad libitum.*

We measured *in sacco* DM digestibility using three fistulated, non-reproductive female reindeer that were fed a pelleted formulation (D Ration; Alaska Pet and Garden, Anchorage, AK), which was based on cereal grains (15.3% corn, 22.5% barley (*Hordeum vulgare* (L.)) and roughage (20.0% alfalfa meal, 21.3% oat hulls (*Avena sativa* (L.)), with protein and sugar concentrates (10.0% soybean (*Glycine max* (L.)) meal, 7.5% molasses) and premixes of minerals and vitamins. This type of formulation has been used for over 12 years to meet requirements for maintenance and growth of captive reindeer at this facility (Barboza and Parker 2006, 2008). Reindeer were routinely weighed on an electronic load scale (± 0.1 kg; Tru-Test Model 703, San

Antonio, TX) at each handling to monitor any changes in body mass associated with changes in feed intake, body condition, and removal of samples from the rumen.

Samples were analyzed for *in sacco* DM digestibility following the protocol of Ihl and Barboza (2007). Samples from the standard reference set (Table 1) were dried and ground in the same manner as for the *in vitro* assay and prepared in triplicate as follows: 2 g of each ground sample were loaded into weighed 5 cm x 10 cm concentrate bags (50 µm pore size), heat sealed, and oven-dried at 50ºC for 24 hours. We then attached sample bags to a rumen suspension device attached to the fistula plug. We used empty sample bags as controls and also included an alfalfa standard in each group of 27 samples. We removed sample bags after 48 hours and gently rinsed the bags under cold water to remove surface residue. The sample bags then underwent an acid-pepsin treatment as described for the *in vitro* procedure before being oven-dried at 50ºC for 48 hours. All samples were assayed in triplicate with one sample in each of three fistulated reindeer.

We measured *in vivo* DM digestibility using five 2-year-old female reindeer that were held in a 1.9-ha pen. Reindeer were fed formulated diets using a Calan Broadbent Feeding System to measure individual intake (American Calan, Inc., Northwood, NH; Mazaika et al. 1988, Thompson and Barboza 2013, 2014) during late summer (27 June to 14 August) and after the breeding season in autumn (27 September to 7 November). We fed two high protein (2.6% N) rations in late summer: D ration (Table 1) and a high fiber ration (47% NDF). We fed the same D ration (Table 1) again in autumn followed by another high fiber (55 % NDF) ration with low protein (1.2% N). We subsampled (70 g) the feed offered each day. Freshly voided feces were collected after each animal had acclimated to the diet for at least 10 days. Replicate samples of feces were frozen, freeze-dried, and ground for analysis. Samples of feed and feces

were analyzed for acid lignin to assess *in vivo* DM digestibility (Barboza and Parker 2006; Barboza et al. 2006).

We tested effects of individual and temporal variation on *in sacco* DM digestibility using alfalfa standards, and on *in vivo* DM digestibility using the D ration diet. We examined how acid-pepsin treatment affected *in sacco* DM digestibility estimates. Finally, we compared *in sacco* DM digestibility with *in vivo* DM digestibility of four mixed diets, and compared *in vitro* DM digestibility with *in sacco* DM digestibility for all samples in the standard reference set (Table 1).

Chemical and Data Analysis

We analyzed each sample in the standard reference set for total N, C, and NDF (Table 1; Barboza and Parker 2006). Dried residues from *in vitro* digestion were assayed for N and C to determine digestibility of each component (Barboza et al. 2006). Dry matter digestibility in filter bags (D_{DMB}) was calculated as the proportional loss of DM:

$$D_{DMB} = (I_{net} - O_{net}) / I_{net}$$

where I_{net} and O_{net} are the respective dry mass of the sample before and after incubation following correction for the change in the mass of an empty bag. Digestibility of a specific nutrient within DM (D_x) was therefore calculated as the proportional loss of that nutrient:

$$D_x = ([I_{net} \times X_w] - [O_{net} \times X_r]) / [I_{net} \times X_w]$$

where X_w and X_r are the concentrations of the component in the DM of the whole sample and the residue respectively. Residual N was corrected for absorption of N from the solution, which was measured as N uptake of cellulose (0.082 g N/100 g DM). Digestibility of dry mass over the whole gut (D_{DML}) was based on the concentration of the indigestible marker lignin:

$$D_{DML} = (F_{Lig} - I_{Lig}) / F_{Lig}$$

where I_{Lig} and F_{Lig} are concentrations of lignin in the DM of ingested feed and feces respectively (Barboza et al. 2009).

All statistical analyses were conducted in Stata 12.0 (StataCorp, College Station, Texas). Tables and figures report means ($\bar{x}$) ± 1 SD. Data that satisfied criteria for normal distribution (Shapiro Wilk test) and homogeneity of variance among groups (Levene's test) were analyzed by parametric approaches including *t*-test, analysis of variance (ANOVA), and linear regression. We used Kruskal-Wallis analysis of variance by ranks to compare digestibilities among incubation times. We used Wilcoxon's signed-rank test to compare N digestibility with DM digestibility.

Results

Method Development

Simple dissolution in acid removed most of the DM during incubation of a mixed diet. Dissolution in pH 5 buffer removed 30 ± 3% of DM when incubated without enzymes and 35 ± 5% of DM when incubated at pH 1 in acid without pepsin over 48 hours. Pepsin activity removed an additional 10 ± 4% of DM when compared with dissolution only in an acid solution. Removal of DM by amylase accounted for an additional 8 ± 2% of DM when compared with dissolution in pH 5 buffer. Removal of DM by cellulase was low (1 ± 1%) when compared with dissolution alone of this diet.

Different incubation times were necessary to achieve DM digestion for the acid-pepsin and amylase-cellulase steps. DM removal by acid-pepsin from ground corn kernels and straw did not change with increased incubation time (corn: $H_2 = 1.69$, $P = 0.43$; straw: $H_2 = 3.20$, $P = 0.20$) but DM digestibility of alfalfa in acid-pepsin increased slightly from 6 to 48 hours (6 hour

DM digestibility = 40 ± 6% vs. 48 hour DM digestibility = 44 ± 8%; H_2 = 6.49; P = 0.04; Fig. 1). We proceeded with the 6 hour incubation in acid-pepsin as a pretreatment for digestion of carbohydrates. Amylase-cellulase digestion of alfalfa and straw did not change with increased incubation time (alfalfa: H_2 = 5.69; P = 0.06; straw: H_2 = 5.42; P = 0.07), but increased greatly with increasing incubation times for corn kernels (24 hour DM digestibility = 76 ± 7% vs. 72 hour DM digestibility = 90 ± 2%; H_2 = 7.20; P = 0.03; Fig. 1). Thus, to accommodate digestion of samples high in starches, we proceeded with the 72 hour incubation with amylase-cellulase.

The order of incubation in acid-pepsin and amylase-cellulase affected the accuracy and precision of DM digestibility estimates of two feed samples (alfalfa and high fiber/high protein feed ration). Samples digested first in acid-pepsin, followed by amylase-cellulase, yielded higher (t_{10} = -3.42; P < 0.01) and more consistent ($F_{5, 5}$ = 5.85; P = 0.04) measures of DM digestibility for alfalfa and high fiber/high protein ration than those incubations that were conducted in the opposite order (alfalfa: 58 ± 0% vs. 51 ± 1%; high fiber/high protein ration: 60 ± 1% vs. 56 ± 3%). Therefore, we proceeded with acid-pepsin incubation as the first stage followed by amylase-cellulase incubation.

In vitro DM digestibility was affected by several physical and chemical properties of the samples. Neutral detergent fiber content was inversely related to *in vitro* DM digestibility with Viscozyme ($F_{1, 91}$ = 235.67; P < 0.01; Fig. 2). Pore size of filter bags also affected digestibility of DM. Samples incubated in concentrate bags (50 µm) were degraded to a greater extent (16 ± 6%) than those incubated in F57 bags (25 µm) when samples in both types of bag were digested according to the same *in vitro* protocol (alfalfa: t_4 = -10.24; P < 0.01; corn: t_4 = -24.71; P < 0.01; straw: t_4 = -14.75; P < 0.01). Sample preparation also affected DM and N digestibility. Dry matter digestibility of freeze-dried forage samples was higher (53 ± 8% vs. 49 ± 7%) but linearly

related to the digestibility of air-dried samples (air-dried DM digestibility [g/g] = 0.67 × [freeze-dried DM digestibility (g/g)] + 0.14; $F_{1, 32}$ = 76.28; P < 0.01; r^2 = 0.71). Nitrogen digestibility of freeze-dried forage samples was also higher (47 ± 5% vs. 40 ± 10%) and linearly related to the digestibility of air-dried samples (air-dried N digestibility [g/g] = 1.57 × [freeze-dried N digestibility (g/g)] -0.35; $F_{1, 7}$ = 16.04; P < 0.01; r^2 = 0.70).

Accuracy of DM Digestibility Methods

In sacco DM digestibility of alfalfa standards without acid-pepsin treatment ranged from 0.39 - 0.77 g/g ($\bar{x}$ ± SD: 0.63 ± 0.08 g/g). Subsequent treatment with acid-pepsin further increased DM digestibility and reduced variation (range: 0.52 – 0.83 g/g; $\bar{x}$ ± SD: 0.71 ± 0.06 g/g). DM digestibility of *in sacco* alfalfa standards were not significantly affected by animal ($F_{2, 15}$ = 1.25; P > 0.05) or time ($F_{11, 15}$ = 1.21; Box's ε = 0.43), even though the fistulated reindeer gained a small amount of mass (4.9 ± 0.6 kg; t_2 = -14.80; P < 0.01) over the experimental period. Therefore, we did not correct *in sacco* DM digestibility for time or animal in subsequent comparisons.

In vivo DM digestibility of mixed diets ranged from 0.63 – 0.91 g/g ($\bar{x}$ ± SD: 0.77 ± 0.08 g/g). *In vivo* DM digestibility did not differ from *in sacco* DM digestibility (*in sacco* slope 95% CI: 0.7 – 1.1; constant 95% CI: -0.1 – 0.2; Fig. 3) for a set of mixed diets (n = 3) after accounting for repeated measures of individual animals over time. *In vivo* DM digestibility was significantly different, however, among animals ($F_{4, 11}$ = 5.57; P = 0.01) and over time ($F_{2, 11}$ = 17.54; Box's ε < 0.01; Fig. 3). Decreases in daily feed intake of the same mixed diet (D ration) from summer to autumn (88.5 ± 6.1 to 55.8 ± 6.4 g/kg$^{0.75}$/d) were accompanied by decreases in DM digestibility from 0.89 ± 0.01 g/g to 0.81 ± 0.05 g/g.

In vitro DM digestibility with cellulase and *in vitro* DM digestibility with Viscozyme were both significant predictors ($F_{1, 27}$ = 39.39 and $F_{1, 28}$ = 91.74, respectively; both $P < 0.01$; Fig. 4) of *in sacco* DM digestibility. The *in vitro* with Viscozyme method was more accurate in predicting *in sacco* DM digestibility than the *in vitro* with cellulase method (Viscozyme $r^2 = 0.77$ vs. cellulase $r^2 = 0.59$; Fig. 4). *In vitro* DM digestibility was lower than *in sacco* digestibility (*IS*), therefore we derived the following equation to predict *in sacco* digestibility from *in vitro* digestibility for cellulase (*IVC*) and viscozyme (*IVV*) respectively:

$IS = 1.10 \times IVC + 0.22$

$IS = 0.87 \times IVV + 0.22$

Variation of *in vitro* DM digestibility of alfalfa standards was much lower than with the *in sacco* method, and was lower still for the *in vitro* method using Viscozyme rather than cellulase ($\bar{x}$ ± SD: 0.58 ± 0.01 g/g for Viscozyme vs. 0.48 ± 0.02 g/g for cellulase).

Digestibility of Specific Nutrients using Viscozyme

Digestibilities of C ranged from 0.03 – 0.87 g/g C while those of N were 0.07 – 0.95 g/g N. Although digestibilities of C were lower than the corresponding digestibility of DM (-0.05 ± 0.01; t = -0.70; $P < 0.01$), digestibilities of N were not significantly different from that of DM (Wilcoxon's signed-rank Z = 1.27; P = 0.21). Digestible N content was related to total N content (Digestible N [g/g] = 0.87 × [Total N (g/g)] – 0.41; $F_{1, 27}$ = 248.23; r^2 = 0.90; $P < 0.01$; Fig. 5) in a linear relationship that indicated digestible N was unavailable below total N concentrations of 0.03 – 0.06 g N/100 g DM. Similarly, the regression relationship between indigestible N in the residue from *in vitro* digestion and the total N in the forage (Indigestible N [g/g] = 0.13 × [Total

N (g/g)] + 0.41; r^2 = 0.17; P = 0.02) predicted a loss of 0.03 – 0.06 g N/100 g DM when total N was zero.

Discussion

DM Digestibility

The concentrations of fiber (NDF) and N in our sample set spanned the range of forage qualities used in north-temperate habitats by caribou, moose (Spalinger et al. 2010) and Sitka black-tailed deer (*Odocoileus hemionus sitkensis* (Merriam, 1898); Hanley and McKendrick 1983). We measured DM digestibility using three different techniques. Digestibility of DM was similar between the *in vivo* and *in sacco* methods. Our estimates of digestion *in sacco* and *in vivo* were similar to published estimates of digestibility for forages of similar composition in studies of other cervids (Spalinger et al. 2010). In comparison with the *in sacco* method, the *in vitro* method provided precise but consistently lower measures of DM digestibility, which can be used to estimate *in sacco* values by correcting for the bias of the *in vitro* procedure.

We relied primarily on the comparison of feed samples measured with both the *in sacco* and *in vitro* techniques because it was not feasible to test every feed sample with the *in vivo* procedure. Digestibility of DM was probably similar between *in vivo* and *in sacco* methods because they both use microbial communities to break down the majority of DM in feeds. These microbes are very efficient in DM digestion for a number of reasons: microbes are present in diverse assemblages (Dehority 2003), able to secrete a wide range of enzymes (Dehority 2003), can penetrate the fiber matrix (Dehority 2003), and can shift enzyme production to match substrates of ingested feeds (Olsen and Mathiesen 1998). In addition, both the *in vivo* and *in sacco* digestibility methods take advantage of intra-animal conditions optimized for extensive digestion where proportions of endogenous enzymes, substrates, and reaction products are

continually being adjusted to maximize reaction speeds. However, exposure to the entire digestive tract and its associated microbial community is limited to the *in vivo* method. Regardless, DM digestibility estimates obtained with the *in sacco* method were similar to those obtained with the *in vivo* method, indicating that *in sacco* estimates were suitable proxies for whole-gut digestibility in caribou.

Differences in digestibility of DM among animal-based and *in vitro* methods was probably due to the inefficiency of purified enzymes in comparison with microbial and endogenous enzymes (López 2005). Most of the DM digestibility in the *in vitro* method was due to solubility, with modest increases in DM digestibility due to purified enzymes. The activities of purified enzymes are limited because they can be more susceptible to interference from plant secondary metabolites (PSMs; Van Soest 1984) and reaction products (Tamminga and Williams 1998). Purified enzymes in an *in vitro* batch reaction are restricted to degrading only those substrates that are easily accessible through diffusion and are not replaced if their activity declines over the course of the incubation. *In vitro* systems employ a narrower range of enzymes to degrade fewer types of substrates than *in vivo* or *in sacco* systems. Indeed, our *in vitro* DM digestibility estimates were higher and more closely matched to those estimates obtained with the *in sacco* method when we included a wider range of carbohydrases (Viscozyme) than just cellulase. Our observation is consistent with other studies that report improved *in vitro* digestibility with a wider range of enzymes (Moughan 1999).

Differences in DM digestibility between *in vitro* and *in sacco* methods were also due, in part, to the different bag types used in each assay. We were restricted to using bags with a larger pore size for the *in sacco* method because using a bag with a smaller pore size may have restricted access to some larger microbes such as *Oscillospira* sp. (40-50 µm; Mackie et al. 2003)

present within the rumen. We chose a smaller pore size bag with the *in vitro* method because we wanted to verify that only degraded particles would leave the bag (Kitessa et al. 1999). Thus, some particles that were not fully degraded may have left the *in sacco* bags, which would have biased samples towards a higher and more variable estimate of digestibility. Loss of undegraded particles undoubtedly occurred even within the smaller-pored *in vitro* bag because we measured significant mass loss even without any enzymes in the buffer solution. However these fine particles would have the greatest surface area and thus the highest potential to be degraded by enzymes in the incubation solution or within the digestive tract of an animal. Differing bag sizes may also have caused disparities between methods by affecting flow of materials through the bag, however we believe this to be minimal because the density of sample material in the 5 × 10 cm concentrate bags was similar to that in the F57 bags (0.05 g/cm^2 vs. 0.03 g/m^2).

Carbohydrate was the most prevalent fraction of DM in our feeds, which was present as starch, hemicellulose and cellulose. Consistently high digestibilities of corn kernels indicated that starches were readily degraded by enzymes *in sacco* and *in vitro*. However, novel structural carbohydrates such as chitins and lichenans in lichens may not be degraded with the *in vitro* method because the purified enzymes included in the assay were not specific to these fibers (Perlin and Suzuki 1962; Svihus and Holand 2000). For example, we tested two samples of lichens (*Cladina* sp. and *Flavocetraria* sp.) using the *in vitro* method, but the DM digestibility of these samples were extremely low (*Cladina* sp.: 0.07 ± 0.01 g/g; *Flavocetraria* sp.: 0.06 ± 0.00 g/g) compared to those estimates measured with the *in sacco* method (*Cladina* sp.: 0.16 ± 0.01 g/g; *Flavocetraria* sp. 0.56 ± 0.08 g/g). Studies of lichen digestion in reindeer suggest that digestion may be achieved by microbial enzymes that are induced after a period of acclimatization (Olsen and Mathiesen 1998; Storeheier et al. 2002). We did not acclimate our

study animals to lichens before measuring the digestibility of these feeds *in sacco*, therefore, *in sacco* lichen DM digestibility estimates were much lower than those estimates measured by others for animals acclimated to lichen (e.g. 0.37 ± 0.13 g/g for *Cladina rangiferina* (L.); Thomas et al. 1984). Lichens are similar in physical structure to mosses, which absorb and adhere to materials within digestive fluids (Ihl and Barboza 2007). Lichens may absorb and retain fluids in a similar manner and, thereby limit the exchange of enzymes and substrates and prevent further dissolution and digestion from these permeable bags.

Application of Method

To maintain consistent estimates of DM and N digestibility, further application of the *in vitro* method should control the pore size of bags; sources and types of purified enzymes; incubation length and order; and the sample drying method. Pore size affects DM digestibility by altering the proportion of particles able to leave the bag. Selecting a bag with the proper pore size requires balancing the need to retain undegraded particles within the bag, yet allowing free diffusion of reactive fluids and enzymes through the bag. We suggest that a bag with a small pore size (25 µm) is more appropriate for the *in vitro* method because enzyme molecules are much smaller than whole microbes and can thus diffuse through smaller pores.

As we discovered when we used a carbohydrase mixture (Viscozyme) rather than a single carbohydrase (cellulase; Fig. 4), types of purified enzymes used in the assay affected DM digestibility (Boisen and Eggum 1991; Moughan 1999). Although inclusion of Viscozyme improved DM digestibility, this may change if commercially available enzyme mixtures are altered. However, the *in vitro* DM digestibility estimates were remarkably consistent for alfalfa standards (SD = 0.01 g/g), indicating that the current enzyme mixture yields consistent results.

Specific lengths of each incubation and order of incubation steps were required to maximize carbohydrate digestion, because degradation of structural carbohydrate may be slow (Barboza et al. 2009). The first incubation in acid probably degraded mixed polysaccharides in the hemicellulose fraction of the plant cell walls, which would have enhanced subsequent digestion with amylase-Viscozyme. By doing so, we allowed enzymes in the second step greater access to the fiber matrix, which resulted in higher and more consistent estimates of DM digestibility. High concentrations of starch also required long incubation times (corn kernels; Fig. 1) to achieve maximum DM digestion of structural carbohydrates after the storage polysaccharides were degraded with amylase.

Drying method affected DM and N digestibility in samples of willows. DM and N digestibility were higher in freeze-dried samples than in air-dried samples, probably because the matrix of the plant remained more open and allowed for increased enzyme access to substrates. Others have also noted an increase in DM digestibility when samples were freeze-dried as compared to air-dried (Dzowela et al. 1995).

Nutrient Digestibility

A major advantage of our *in vitro* method is that it allows for measurement of the digestibility of specific nutrients in addition to DM (DeGabriel et al. 2008). Dry matter digestibility is the most common measure of digestibility, yet DM is not *per se* a nutrient – rather, it is a category which encompasses all other nutrients, each of which may or may not have the same digestibility as DM. Digestibility of N and the digestible concentration of N in forages are of particular interest to herbivore ecologists because population growth may be limited by N intake (Batzli 1983; DeGabriel et al. 2009; McArt et al. 2009). Digestible N is easily measured with this technique (DeGabriel et al. 2008) because we do not use microbes to digest the forage

and because we can thoroughly wash the residue to remove reactant enzymes. Our method indicates that digestible N increases with total N content of the forage but the relationship suggests that N is unavailable to digestive enzymes as total N falls below 0.03 - 0.06 g/g DM. Poor digestibility of N is due to incorporation of N in fiber and inhibition of enzymes by PSMs. Digestible N was lowest for woody browse, probably due to a high PSM content. These samples were collected in late in the growing season when PSM content may be at its peak (McArt et al. 2009).

Our *in vitro* digestibility method can be used to measure digestibility of other nutrients or to measure the effects of digestion on substrates without contamination by endogenous or microbial residues from herbivores. In particular, this method may be expanded to measure effects of digestion on stable isotope values of forages to improve estimates of fractionation (changes in stable isotope values between forages and feces or forages and animal tissues). Such measurements taken in the past have been confounded with microbial or endogenous contamination of feces (Gustine et al. 2011; Gustine et al. 2014). In addition, it is also possible to measure digestibility of other nutrients that may limit populations, including minerals. Our method lacks any lipid-digesting enzymes, so samples high in lipid content have the potential to block pores in the bags and prevent the flow of enzymes and substrates (Tamminga and Williams 1998). Such samples may need to be treated with a lipase (Moughan 1999) or a solvent to remove the lipid fraction. Generally, however, lipids are present in very low abundance in herbivore diets but are readily digested by the animal or its microbes.

Conclusion

Measurements of forage digestibility for wild herbivores are most commonly made using variable and costly animal-based techniques that are not always suited to monitoring. Indeed, *in*

vivo DM digestibility in our study varied by as much as 0.08 g/g over the course of a few months. *In vitro* DM digestibility was much more consistent and is easily corrected to more realistic *in sacco* DM digestibility values. It is also possible to measure the digestibility of individual nutrients with our method, and we showed that no net nitrogen would be available to the animal below total nitrogen concentrations of 0.03 – 0.06 g N/g DM.

Our *in vitro* method is also more practical than animal-based options for routine application in a laboratory setting. The *in vitro* method is comparatively cheap, and we calculated that consumables for each sample would cost $4.50 (triplicate assays) under current market conditions. The initial investment in an incubation system for this method ($2,000 - $5,000) is considerably less than the annual cost of operating and maintaining fistulated animals ($1000 – $2000/animal). Both systems require balances, ovens, mills and labor to prepare and process samples. While the time required for processing samples with the *in vitro* method is roughly the same as with the *in sacco* method (i.e., one week), four times more samples can be run in the same time frame. However, we determined that bag pore size, enzyme mixture, incubation length, and sample drying method can affect *in vitro* estimates, and laboratories should control for these factors when possible to obtain consistent results. Nevertheless, our *in vitro* method is more suited for monitoring forage quality because it can generate accurate, precise, biologically relevant, and cost-effective estimates of forage quality for herbivores.

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Table 1.1. Classification and composition of samples (% dry matter) digested using *in sacco* and *in vitro* techniques. Measurements denoted with n.a. were insufficient for analysis.

Forage Class	Sample	NDF[a]	N[b]	C[c]
Mixed Diet	D ration	37	2.7	44.3
	High fiber/high protein ration	57	2.7	42.0
	Low fiber ration	37	1.9	43.0
	Medium fiber ration	40	1.7	42.5
Graminoid	Brome Hay (*Bromus* sp.)	72	1.1	45.9
	Carex aquatilis Kuparuk River	63	1.9	48.1
	Carex aquatilis Prudhoe Bay	64	2.4	47.8
	Eriophorum vaginatum Kuparuk River	71	1.7	49.2
	Eriophorum vaginatum Prudhoe Bay	68	1.7	48.2
	Straw (*Triticum* sp. x. *Secale* sp.)	80	0.8	44.1
Browse	*Betula papyrifa* leaves – green	59	1.9	47.2
	Betula papyrifa leaves – yellow	59	0.8	48.9
	Picea sp. needles	47	1.0	49.1
	Salix alaxensis leaves	43	1.4	45.5
	Salix barclayi winter twigs	56	1.0	52.2
	Salix pulchra leaves	34	1.9	52.3
Forb	Kelp (*Ascophyllum nodosum*)	43	1.1	36.0
	Epilobium angustifolium	44	1.6	45.6
	Equisetum sp.	60	1.9	36.8
	Alfalfa (*Medicago sativa*)	55	3.1	43.6
	Pedicularis spp.	57	2.0	48.5
Fungi	*Boletus* sp.	n.a.	3.1	42.8
	Puffball mushrooms (*Lycoperdon* sp.)	n.a.	8.0	35.3
Plant parts	Barley (*Hordeum vulgare*) kernels	37	1.9	44.8
	Beet (*Beta vulgaris*) pulp	49	1.4	43.6
	Corn (*Zea mays*) cobs	80	0.7	44.1
	Corn (*Zea mays*) kernels	14	1.2	45.2
	Oat (*Avena sativa*) hulls	60	1.2	45.9
	Soybean (*Glycine max*) hulls	68	1.9	44.0
	Rice (*Oryza* sp.) hulls	86	0.5	39.2
	Viburnum edule fruit	38	0.9	48.7

[a]Neutral detergent fiber
[b]Nitrogen
[c]Carbon

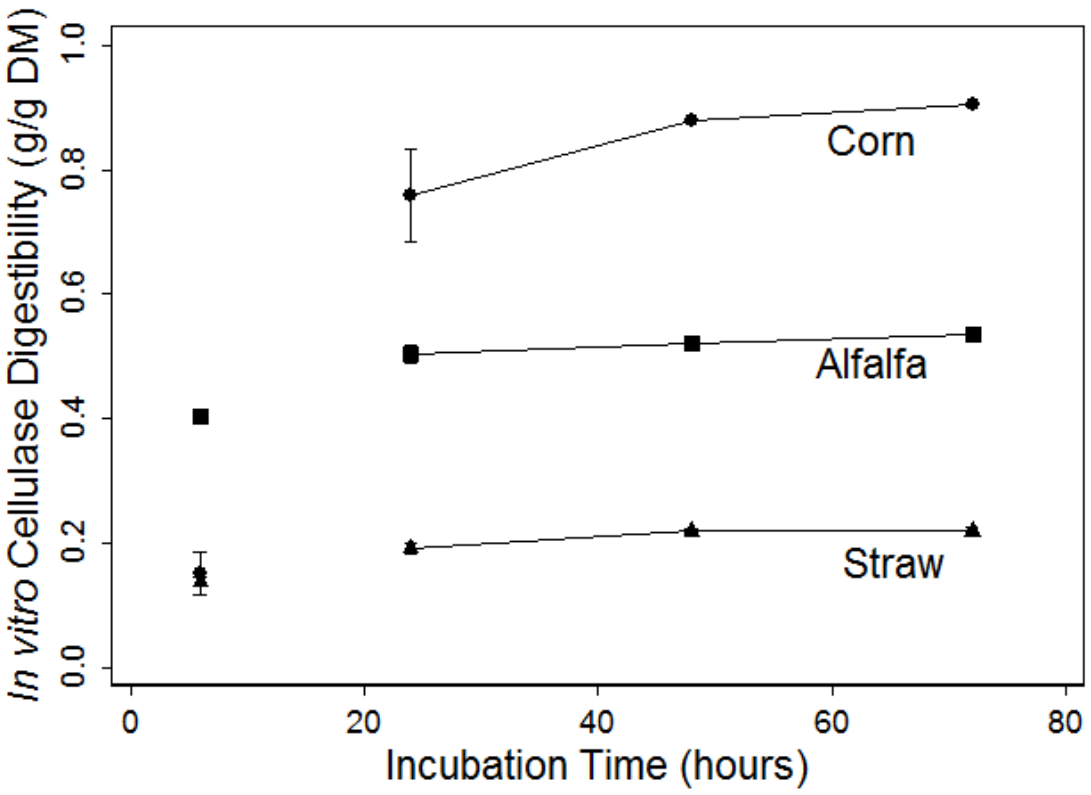

Figure 2.1. The change in the *in vitro* dry matter (DM) digestibility of alfalfa (*Medicago sativa*), corn (*Zea mays*), and straw (*Triticum* sp x *Secale* sp.) with increasing incubation time with acid-pepsin (6 hours), followed by amylase-cellulase (24-72 hours).

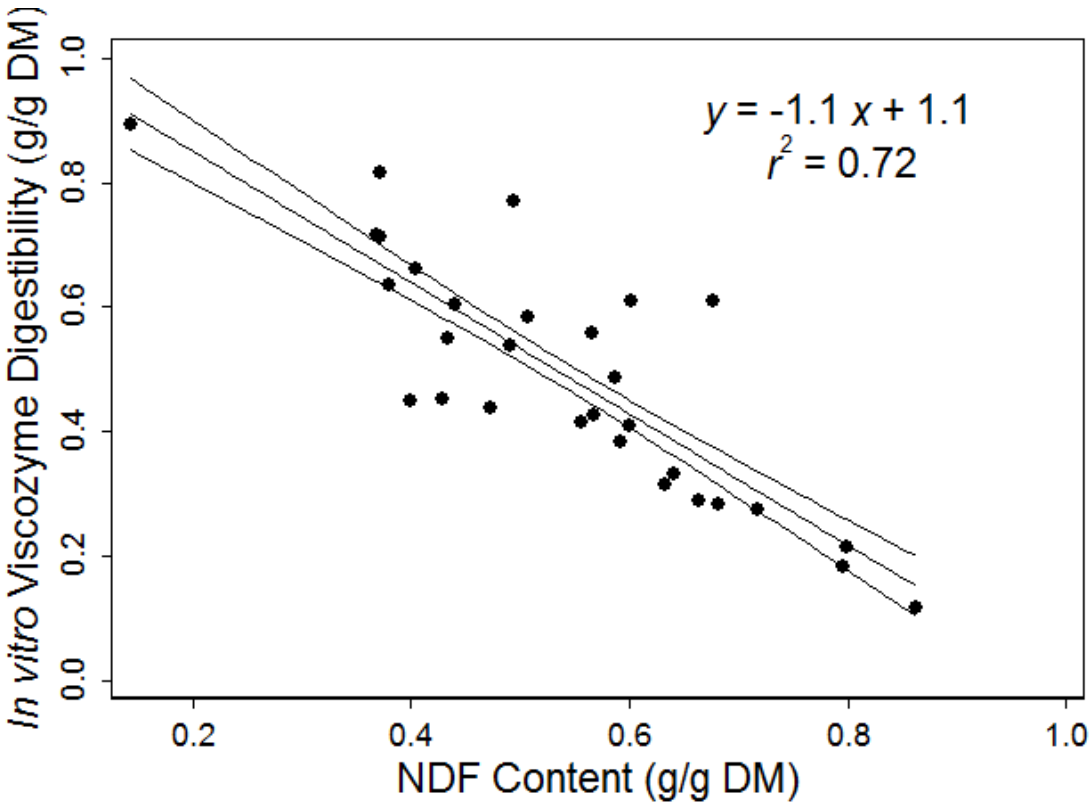

Figure 2.2. The relationship between dry matter digestibility of standard reference samples incubated using the final *in vitro* digestibility protocol and neutral detergent fiber (NDF) content.

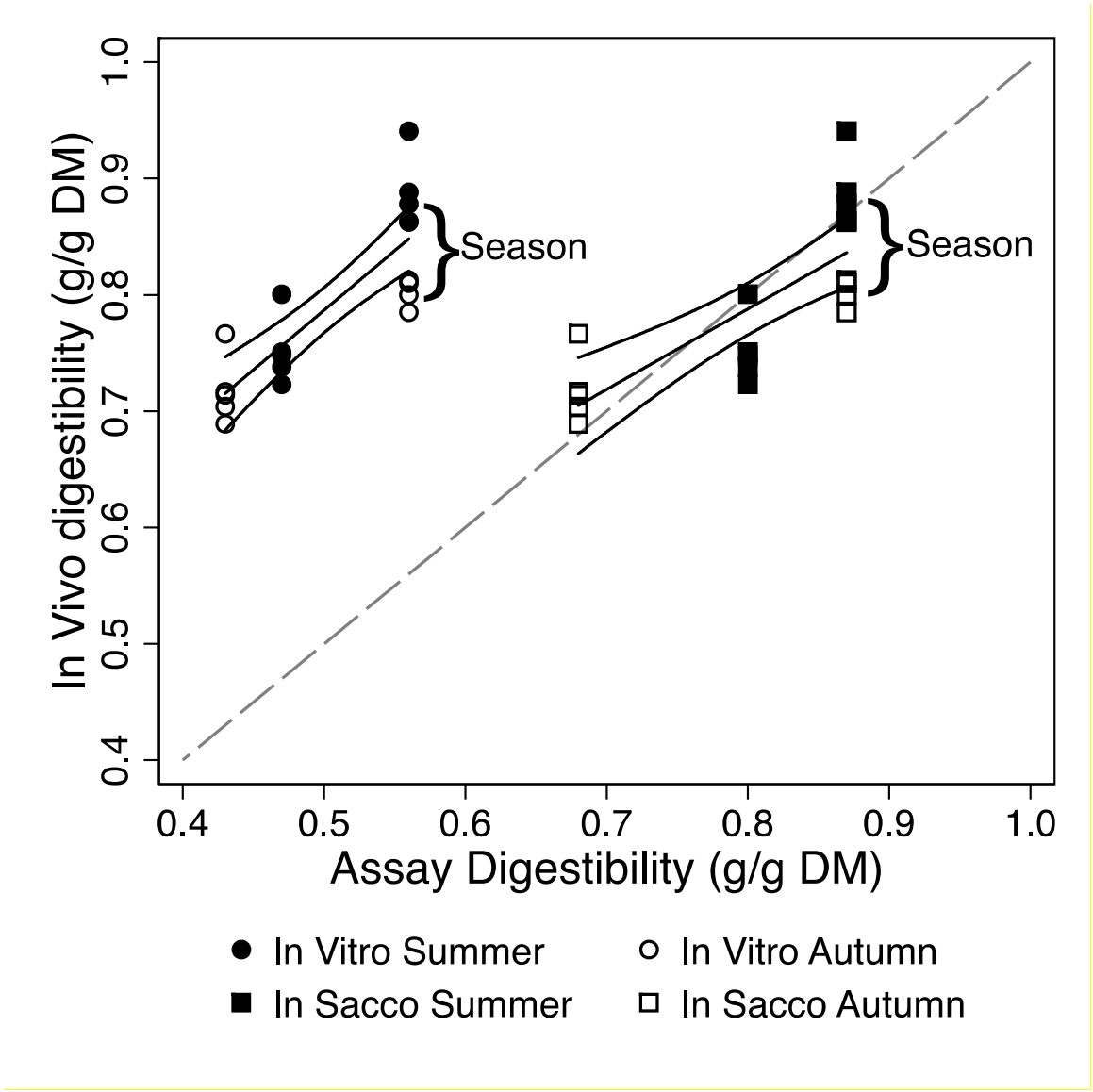

Figure 2.3. Comparison between measures of dry matter (DM) digestibility of animal feed rations digested in filter bags (*in vitro* and *in sacco*) against *in vivo* DM digestibility measured over the whole tract. Points represent individual animals and the solid line represents unity.

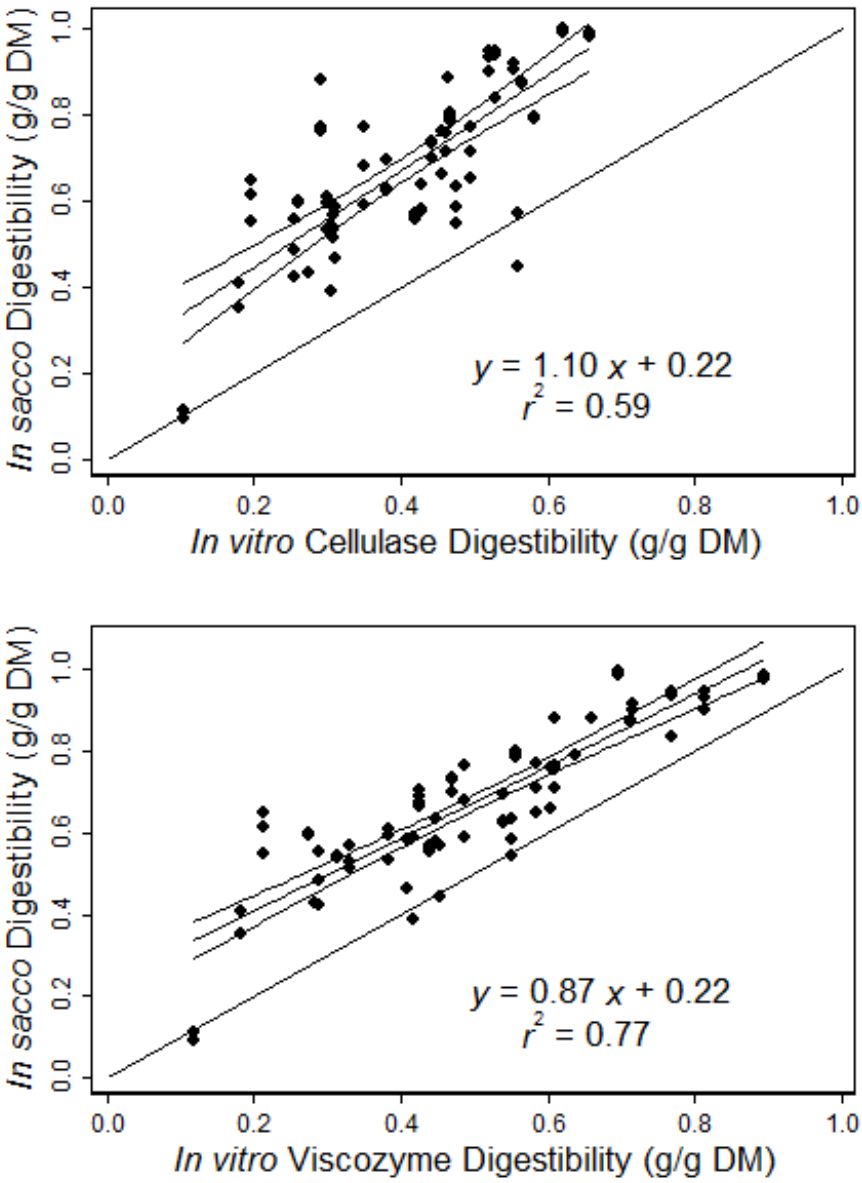

Figure 2.4. Comparison of *in sacco* DM digestibility with *in vitro* DM digestibility using either a) cellulase, or b) Viscozyme. Points represent samples from the standard reference set.

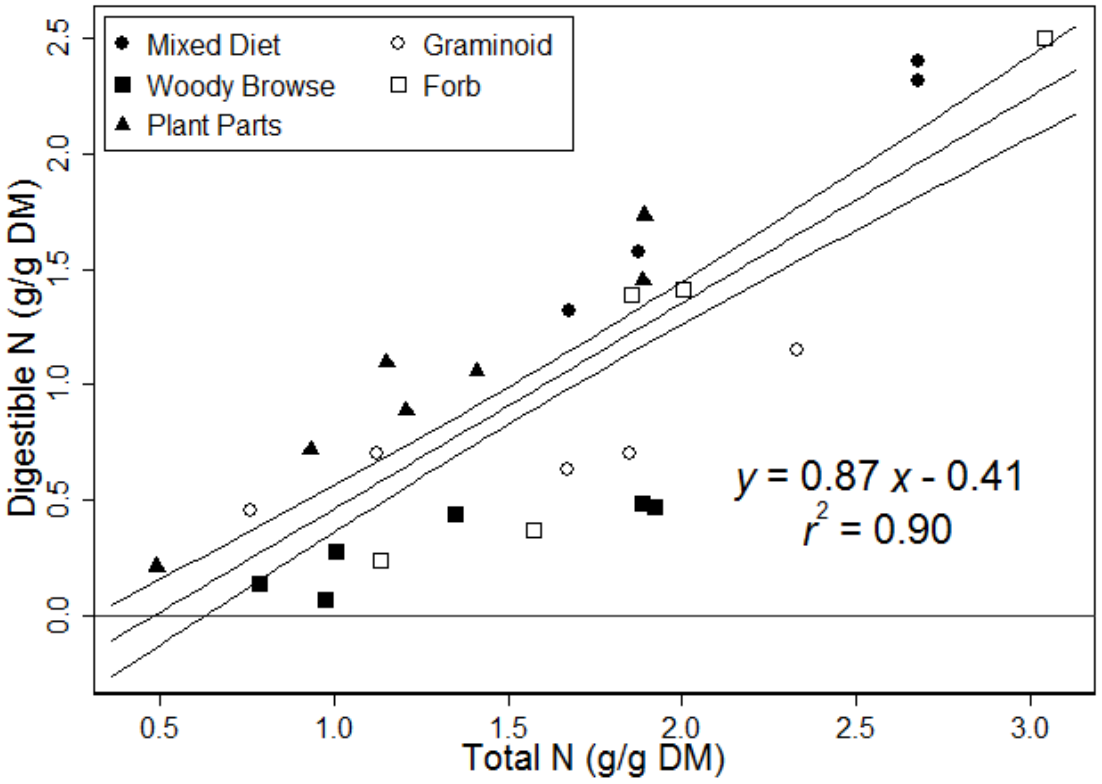

Figure 2.5. Relationship between concentrations of digestible N and total N of standard reference feeds. Digestible N was calculated from the N content of the digested residues.

CHAPTER 3: ASSESSING NUTRIENT AVAILABILITY FOR ARCTIC CARIBOU: INTERACTIONS AMONG NUTRIENT, PLANT FIBRE, PHENOLIC COMPOUNDS, AND STABLE ISOTOPE RATIOS[2]

Summary

1. Arctic herbivores such as caribou are often limited by content of digestible nutrients in forage plants, which can vary widely among species and regions through the course of the year. The selection of forage plants may be assessed with stable isotope techniques. However, our knowledge of how fine-scale digestible nutrient content and stable isotope values vary across caribou summer ranges is limited.

2. We examined how plant fibre and phenolic compounds interacted with nutrient availability through the summer for seven caribou forage species growing along a 200-km transect through the range of the Central Arctic caribou herd on the North Slope of Alaska. We measured total nitrogen (N), carbon (C), and gross energy as well as $\delta^{13}C$ and $\delta^{15}N$ content before and after simulated digestion.

3. We found that $\delta^{15}N$ was best suited to tracking diet among arctic herbivores because $\delta^{13}C$ exhibited spatiotemporal differences that were as large as the variation among forage classes. Digestive processes may also bias isotope ratios in tissues and feces because isotopic differences among the diet, indigestible fraction, and the digestible fraction of forage plants

[2] Prepared for submission to Functional Ecology as VanSomeren, L. L., P.S. Barboza, D. D. Gustine, and M. S. Bret-Harte. Assessing nutrient availability for arctic caribou: interactions among nutrient, plant fibre, phenolic compounds, and stable isotope ratios.

were increased when they had either very low (<52.6% N) or very high (>37.4% C) digestibilities.

4. Digestible N has a greater potential than digestible energy for limiting growth of caribou populations because contents of this nutrient generally failed to meet minimum reproductive requirements (1.66 g/100 g digestible N) except during the first 30 days of plant growth. Digestible energy was generally sufficient for both reproductive (10.49 kJ/g DM) and maintenance (9.68 kJ/g DM) requirements of caribou for most of the growing season.
5. Graminoids provided low but predictable supplies of N for caribou. Shrubs provided the greatest potential for gaining digestible N, but fibre and phenolics caused wide variation in digestible N among shrub species and seasons. Digestible nutrients available to caribou may be strongly influenced by selection of foraging locations, forage plant species, and plant parts across the season.

Introduction

Herbivore populations are often limited by the ability of individual animals to obtain and retain nutrients needed for their survival and reproduction (Barboza et al. 2009, Parker et al. 2009). Survival requires large quantities of energy in the form of fat, carbohydrates, and protein, which animals use to support daily metabolic functions. Arctic herbivores often guard against shortages in energy intake during the long winter months by storing energy in the form of fat reserves. These stored fat reserves affect reproduction by altering fertility rates (Thomas 1982, Allaye-Chan 1993) and reproductive success (Crête and Huot 1993) in females. Reproduction is also affected by protein stores because large amounts of protein are required to form offspring and reproductive tissues in winter when protein intake is at an annual minimum (Barboza and

Parker 2008, Gustine 2010). While nutrient content of forage plants is lowest in winter, protein content, in particular, is much lower than energy content. Thus, the net cost of reproduction above the maintenance level is greater for protein than for energy in arctic herbivores such as caribou (Barboza and Parker 2008). The relative impacts of dietary intake of energy and protein on productivity of arctic herbivore populations is poorly documented, but insights may be obtained from comparing animal nutrient requirements with the availability of nutrients in forage plants.

Nutrient contents in forage plants are often low and variable, forcing herbivores to adopt a wide range of strategies to cope with nutrient shortages. Herbivores can migrate to more favorable locations (Hebblewhite et al. 2008, Sawyer and Kauffman 2011), select plant parts or species of high quality (White 1983), eat more forage (Barboza et al. 2006, Thompson and Barboza 2013), adjust their rates of N loss (Parker et al. 2005), limit activity in order to reduce energy demands (Cuyler and Øritsland 1993), or apply a combination of these behavioral and physiological responses. Despite attempts to maximize nutrient intake, many populations of herbivores are still limited by the ability of individuals to gain nutrients from the environment (White 1993, DeGabriel et al. 2009, McArt et al. 2009).

Highly seasonal growth of plants in the Arctic constrains the window of opportunity for herbivores to forage and acquire the necessary nutrients. Opportunities for selecting high-quality forages are further limited by low levels of aboveground biomass and low forage species diversity found in arctic tundra ecosystems as compared to more temperate ecosystems (Chapin et al. 2011). Seasonal timing of foraging also affects nutrient acquisition because phenological changes in arctic plants compress the window of nutrient availability for herbivores. Nutrient contents decline as leaves expand in spring, and increasing contents of non-nutrient plant matter

dilute the digestible components as the leaves mature and progress to senescence. Nutrient availability is further reduced during senescence by mobilization of sugars and protein from leaves to plant storage organs to support resumption of growth in the following year (Chapin et al. 1980). Additionally, much of the tundra is composed of small shrubs that are defended to varying degrees by plant secondary metabolites (PSMs). PSMs reduce the availability of dietary N to herbivores by directly binding to plant proteins and digestive enzymes (Robbins et al. 1987, Spalinger et al. 2010), or by increasing the loss of N as conjugated nitrogenous substances during detoxification of the body (Au et al. 2013).

Nutrient intake by herbivores is affected by diet composition because forage species vary in contents of both total nutrients and substances such as PSMs and fibre that interfere with digestion. Indeed, caribou are known to vary their diets extensively throughout the course of the summer (Thompson and McCourt 1981, Russell et al. 1993). Diet composition of caribou may be altered in the future by projected increases in shrub abundance due to a changing climate (Sturm et al. 2001, Epstein et al. 2004, Tape et al. 2012). Changes in the amount of shrubs in the diet will likely affect arctic caribou (Thompson and Barboza 2014), but because the nutrient content available in these forage plants is not clear, it is difficult to predict the nutritional effects, if any, to caribou.

Stable isotopes of C and N have been used to measure dietary intake in wild herbivores (Codron and Codron 2009), but their use for fine-scale dietary analysis in the Arctic has been problematic. Proportions of diet composed of each forage species are calculated using mixing models, and these estimates are more precise and accurate when forage species have large and distinct differences in stable isotope values. In fact, most dietary analyses for herbivores rely on assigning diet to C3 deciduous shrubs and C4 grass categories because of the large differences in

^{13}C between these groups (Chapin et al. 2011). In northern ecosystems, however, C4 plants are absent (Öpik and Rolfe 2005), leaving only small differences in ^{13}C among the C3 forage plant species to resolve the contributions of different plant groups to the diet. Nevertheless, both ^{13}C and ^{15}N have been used successfully for broad-scale dietary analysis in northern herbivores (Finstad and Kielland 2011, Kristensen et al. 2011), although less frequently than in temperate and tropical systems (e.g., Codron and Codron 2009, Wittmer et al. 2010, Codron et al. 2011b).

Mixing models also require adjusting diet components for fractionation - a phenomenon observed when isotope values of animal tissues are elevated above those of their diet sources due differences in reaction rates among isotopes as they go through the many physical and chemical changes between digestion and incorporation into animal tissues (Fry 2006). Most studies use constant values (e.g., +3‰ for $\delta^{15}N$) between forage plants and herbivores, but recent work has suggested that diet-tissue fractionation values may in fact be influenced by the quality of forage sources, because differing fractions of ^{13}C and ^{15}N are presented to the herbivore according to the digestibility (Codron et al. 2011a) and/or protein quality (Florin et al. 2011) of those food items. Accurate diet-feces fractionation values of ^{15}N are difficult to obtain due to contamination of ^{15}N-enriched microbial and endogenous compounds in feces (Gustine et al. 2014a). Adjusting diet sources with correct fractionation values will improve fine-scale mixing model accuracy and precision, and may be especially important in Arctic ecosystems that are already limited by smaller isotopic differences between forage plant classes compared to temperate and tropical ecosystems.

Our objectives in this study were to examine fine-scale seasonal, regional, and species-specific patterns in contents of digestible N, digestible energy, and stable isotope ratios of C and N in forage plants growing on the summer range of the Central Arctic caribou herd on the North

Slope of Alaska, in order to determine the magnitude of nutrient supply to these animals as well as the best isotopic methods to track dietary sources of these nutrients. We collected several forage species representing the bulk of the caribou diet along a large spatial and temporal gradient, and assessed these forage species for nutrient, fibre, and phenolic compound contents in order to evaluate how nutrient availability is limited by these anti-nutritional compounds. Reductions in N due to concurrent effects of fibre and PSMs have previously been difficult and costly to measure, however recent advances in techniques have allowed for rapid and easy measurement of digestible N (DeGabriel et al. 2008, VanSomeren et al. in review). We used these techniques to compare digestible nutrient contents to nutrient requirements of reproductive and barren female caribou to assess the implications of variable plant quality on their productivity. Stable isotope ratios of C and N in forage plants were measured before and after simulated digestion to determine which of these isotopes would be most useful for caribou diet estimation.

Materials and Methods

Study Area and Sampling Design

This study was conducted in the summer range of the Central Arctic caribou herd on the North Slope of Alaska (Fig. 1) from 2011 to 2013 (Whitten and Cameron 1980, Arthur and Del Vecchio 2009). We sampled 9 sites spread evenly along the Dalton Highway from the Kuparuk River to Prudhoe Bay (Fig. 1). Sites were classified into 3 ecoregions: Brooks Range, Arctic Foothills, and Coastal Plain according to Gallant et al (1995). Caribou in this herd typically winter within or south of the Brooks Range (Gustine et al. 2014b) before migrating northwards through the Brooks Range and Arctic Foothills to reach the calving grounds in the Coastal Plain ecoregion in early summer (Arthur and Del Vecchio 2009). After giving birth, females typically

spend the rest of the summer in the Coastal Plain ecoregion before slowly migrating southwards again through the Arctic Foothills and Brooks Range during the fall breeding season (Cameron et al. 1979, Jakimchuk et al. 1987). Although bulls also use the Coastal Plain ecoregion, they generally have a more southerly distribution than females during the growing season (Cameron and Whitten 1979, Jakimchuk et al. 1987).

Samples of six preferred forage species (*Carex aquatilis*, *C. bigelowii*, *Eriophorum vaginatum*, *Pedicularis* spp., *Salix pulchra*, and *S. richardsonii*; Thompson and McCourt 1981) were collected, when present, every two weeks from late May - late September. In addition, we collected samples of *Betula nana* in 2012 and 2013, because, although this species does not make up a large part of North Slope caribou diets at present (Russell et al. 1993), it is increasing in abundance throughout the Arctic (Sturm et al. 2001, Myers-Smith et al. 2011, Kaarlejarvi et al. 2012). Indeed, another shrub birch, *B. glandulosa*, makes up a significant part of caribou diets in Quebec (Crete et al. 1990). Forage plants were sampled to mimic caribou browsing and grazing – i.e., for deciduous shrubs, easily accessible leaves and twigs were stripped off, while forbs and graminoids were clipped at ground level.

Forage samples were transferred to paper bags and air-dried at ambient temperature (0-22º C) in the field, then air-dried to constant mass in a forced-air oven at 50-55º C when samples were returned to the laboratory, within 2-6 days of collection. A small subsample (approximately 70 g) of deciduous shrubs was immediately frozen in the field and freeze-dried upon return to the lab (Labconco Model 7755044, Kansas City, Missouri, USA) to test for the presence of PSMs. Dried samples were ground through a #20 mesh (1.27 mm) in a Wiley mill (Thomas Scientific, Swedesboro, New Jersey, USA) or a centrifugal mill (Retsch ZM 200, Haan, Germany).

Laboratory Analyses

We measured the total N content in 771 forage samples with an elemental analyzer (CNS2000, LECO, St. Joseph, Michigan, USA). Phenology curves of total N vs. ordinal day were established for each species × site × year combination, and we used these curves to select representative samples for further analysis from 3 key time periods: early season (peak N content), mid-season (peak biomass), and late season (last sampling date). These samples were analyzed for C, dry matter content (DM), and N digestibility (g digested/g whole) by analyzing the nutrient content of digested residues obtained using an *in vitro* digestibility method with purified enzymes (DeGabriel et al. 2008), which has been validated for caribou (VanSomeren et al, in review). Digestible N content was calculated by multiplying N digestibility by total N content for each forage sample. PSM content was measured as total phenolic compounds by reaction of extracted phenolics with Folin-Ciocalteu reagent, and expressed in equivalents of gallic acid as a standard phenol (mg Gallic Acid/g DM; Singleton et al. 1999). Gross energy content was determined using an adiabatic bomb calorimeter (Parr Instruments, Boleen, Illinois, USA; Barboza et al. 2009). Digestible energy content was calculated as the product of the gross energy content and DM digestibility.

The $\delta^{15}N$ and $\delta^{13}C$ of forage samples (‰) from the early season and late season subsets in 2011 (all species) and in 2012 (deciduous shrubs species only) were analyzed with a Europa Scientific 20-20 Continuous Flow IRMS (Europa Scientific, Chestershire, UK) at the Alaska Stable Isotope Facility. In addition, residues from the *in vitro* digestibility method were analyzed for $\delta^{15}N$ and $\delta^{13}C$ after being extracted with hot water and dried (Gustine et al. 2014a). Fractionation between diet-indigestible fraction ($F_{diet\text{-}indigestible}$) for $\delta^{15}N$ and $\delta^{13}C$ was calculated as:

$F_{diet\text{-}indigestible} = \delta X_{residue} - \delta X_{whole\ plant}$ (eqn. 1)

Fractionation of the diet-digested fraction for $\delta^{15}N$ and $\delta^{13}C$ was calculated by converting each isotope measurement to mass ratios (g isotope/g element) and then multiplying this value by the N and C content (g element/g dry matter), respectively, of whole plants and indigestible residues to obtain the content of ^{15}N, ^{14}N, ^{13}C, and ^{12}C on a dry matter basis. Differences in the content of each isotope between indigestible residues and whole plants were used to calculate mass ratios and $\delta^{15}N$ and $\delta^{13}C$ values of digested fractions, which were then converted into delta notation.

Statistical Analyses

All analyses were conducted in Stata 12.0 (StataCorp, College Station, Texas, USA). Forage species were grouped to facilitate comparisons among plant functional groups: graminoid (*C. aquatilis*, *C. bigelowii*, and *E. vaginatum*), deciduous shrubs (*B. nana*, *S. pulchra*, and *S. richardsonii*), and forb (*Pedicularis* spp.). Groups of data are summarized as mean ± SD where indicated. We used $P < 0.05$ as the criteria for significance of α in all comparisons. Bonferroni corrections were applied to determine the significance of multiple post-hoc comparisons.

We used robust ordinary least-squares linear regression models (OLS) to describe spatial and temporal variation among plant types. Predicted values from OLS models are presented as mean ± SE. Models were run for each of the following dependent variables: total N, N digestibility, digestible N, gross energy, DM digestibility, and digestible energy content. Each model included plant group, ecoregion, and ordinal date as independent variables. We also tested the following interactions: plant group × ordinal date, plant group × ordinal date^2, plant group × ecoregion, ecoregion × ordinal date, ecoregion × ordinal date^2, and ordinal date^2. We chose to assess non-linear terms in the models because preliminary analysis of data showed strongly non-linear patterns of nutrients according to ordinal date. ANOVA models were used to examine

spatial, temporal, and species-specific changes in values for $\delta^{15}N$ and $\delta^{13}C$, with species, ecoregion, and subset (early season or late season) as fixed factors. We tested values of $\delta^{15}N$ and $\delta^{13}C$ for interactions of species × subset, species × ecoregion, and ecoregion × subset. Values of $\delta^{15}N$ and $\delta^{13}C$ were log transformed using the lnskew procedure in Stata (Zar 1999) to meet assumptions for normality and tested using the Shapiro-Wilk procedure. Corrected Akaike's Information Criterion (AICc) was used to compare both ANOVA and linear regression models with different variables and their interactions to select a final model with the lowest AICc score (Anderson 2008). The significance of each parameter was checked with Wald tests.

In order to compare seasonal differences among nutrient contents, we used the regression models to compare ecoregions and species at the beginning of the growing season (ordinal day 150) and at senescence (ordinal day 270). However, where ecoregion differences occurred, at the beginning of the growing season we compared the Coastal Plain ecoregion to the Arctic Foothills and Brooks Range ecoregions at ordinal day 170 because no forages were present on the Coastal Plain ecoregion until this date. Similarly, end-of-season comparisons for *B. nana* and *Pedicularis* spp. were made at ordinal day 240 because that was the last sampling date that these species were collected before senescence. We used non-parametric comparisons (Kruskal-Wallis test, Wilcoxon rank sum, and Spearman's rho) to evaluate variation in phenolic content because this variable was not normally distributed. Preliminary analyses suggested species-specific differences among certain plant groups so we also used Kruskal-Wallis tests to evaluate these subtle differences, because nutrient contents were not normally distributed. Linear OLS regressions were used to examine the effect of N and C digestibility on isotopic fractionation, and to develop predictive relationships for digestible N in relation to contents of total N, ADF (acid detergent fibre) and phenolics for each forage species. Paired *t*-tests were used to determine

significance of fractionation between diet, indigestible residues, and digested fractions for each forage species.

Results

Nitrogen Dynamics

The greatest single factor causing variation among most nutritional variables was ordinal date, and total N varied significantly according to ordinal date as well ($F_{2, 756} = 33.41$; $P < 0.01$; Fig. 2). Plant groups also varied in total N, and differences in total N between groups were greatest at the onset of the growing season when graminoids were significantly lower than deciduous shrubs species and the forb (Table 1). Plant groups declined in total N content at different rates so that differences between groups were smaller by senescence though deciduous shrubs were still higher in total N than both graminoids and the forb (Table 1). Total N content also varied between ecoregions as a function of ordinal date ($F_{2, 756} = 17.09$; $P < 0.01$) and was greater in the Coastal Plain than either the Arctic Foothills or the Brooks Range throughout the growing season ($F_{1, 756} \geq 12.70$; $P < 0.01$). Predicted values for total N at the onset of the growing season on the Coastal Plain (ordinal date 170) were 2.86 ± 0.05 g/100g DM, whereas total N in the Arctic Foothills and the Brooks Range was only predicted at 2.54 ± 0.03 g/100g DM and 2.53 ± 0.03 g/100g DM, respectively, on the same ordinal date.

Nitrogen digestibility varied among plant groups as a function of ordinal date ($F_{2, 327} = 5.13$; $P = 0.01$). Graminoids began the growing season with a higher N digestibility than deciduous shrubs (Table 1), but declined at a faster rate so that the two forage groups were not different in N digestibility at the end of the growing season (Table 1). The forb *Pedicularis* spp. remained highest in N digestibility throughout the growing season ($F_{1, 327} \geq 8.76$; $P < 0.01$), but

declined 19% from the onset of the growing season to senescence (Table 1). Ecoregion was not a significant contributor to N digestibility in the best model (Appendix III).

Nitrogen digestibility was reduced by the presence of fibre and phenolic compounds, which interfered with nitrogen digestion. Nitrogen digestibility decreased with increasing ADF content ($F_{1, 307} = 42.40$; $P < 0.01$; $R^2 = 0.15$; N digestibility = -1.10×ADF content (g/g DM) +0.79) in all forage species. Nitrogen digestibility also decreased with increasing phenolic content in *B. nana* in 2012 (Spearman's rho = -0.78; $P < 0.01$), but increased with increasing phenolic content in 2013 (Spearman's rho = 0.61; $P = 0.02$). Within deciduous shrub forage species, *B. nana* was much lower in N digestibility than the two willows (*B. nana*: 0.24 ± 0.13 g/g N; *S. pulchra*: 0.40 ± 0.15 g/g N; *S. richardsonii*: 0.45 ± 0.12 g/g N; Kruskal-Wallis test; $P < 0.01$).

In order to determine the magnitude of nitrogen supply that would actually be available to animals after interference from fibre and phenolic compounds, we determined the digestible N content of forage plants. Digestible N content varied among plant groups as a function of ordinal date ($F_{2, 318} = 7.54$; $P < 0.01$; Fig. 3). The forb *Pedicularis* spp. contained the highest digestible N contents at the onset of the growing season (Table 1), however this forb's digestible N content declined over the course of the growing season so that it was not different from graminoids in digestible N content by the time it senesced (Table 1). Deciduous shrubs, despite having higher total N contents, were not different from graminoids in contents of digestible N as a function of ordinal date (Table 1) due to reductions caused by fibre and phenolic compounds. Digestible N contents, unlike total N contents, did not vary between ecoregions ($F_{2, 318} = 2.34$; $P > 0.05$) even though ecoregion was selected in the final model (Appendix III). Among deciduous shrubs, *B. nana* was again an outlier, because it contained much lower contents of digestible N than the

willow species (*B. nana*: 0.54 ± 0.48 g/100 g DM; *S. pulchra*: 1.02 ± 0.62 g/100 g DM; *S. richardsonii*: 1.10 ± 0.53 g/100 g DM; Kruskal-Wallis test; $P < 0.01$).

Energy and Dry Matter Digestibility Dynamics

Gross energy content varied among plant groups as a function of ordinal date ($F_{2, 204} = 9.61$; $P < 0.01$). Deciduous shrubs were highest in gross energy content compared to graminoids and the forb at the onset of the season (Table 1). Gross energy content in forage plants declined at different rates throughout the growing season so that by senescence, gross energy content was greatest in deciduous shrubs, intermediate in graminoids, and lowest in the forb (Table 1). Gross energy content varied among species of deciduous shrubs (Kruskal-Wallis test; $P < 0.01$): *B. nana* contained the highest gross energy content (20.89 ± 0.47 kJ/g DM), whereas *S. pulchra* was intermediate (19.56 ± 0.43 kJ/g DM) and *S. richardsonii* was lowest (18.19 ± 0.83 kJ/g DM). Gross energy content did not vary by ecoregion ($F_{2, 204} = 1.88$; $P > 0.05$) even though ecoregion was a significant contributor in the final model (Appendix III).

Dry matter digestibility varied among plant groups as a function of ordinal date ($F_{2, 376} = 18.27$; $P < 0.01$). Plant groups declined in DM digestibility over the growing season at different rates (Fig. 4). Dry matter digestibility was highest for the forb *Pedicularis* spp. (Fig. 4; $F_{1, 376} \geq 28.08$) and declined 9% from plant emergence to senescence (Table 1). Dry matter digestibility was intermediate for deciduous shrub species (Fig. 4; $F_{1, 376} \geq 4.17$) and declined 5% from plant emergence to senescence (Table 1). Graminoids had the lowest DM digestibility (Fig. 4; $F_{1, 376} \geq 4.17$) and declined 16% from plant emergence to senescence (Table 1). Dry matter digestibility varied significantly among deciduous shrub species (Kruskal-Wallis test; $P < 0.01$): *S. richardsonii* was the most digestible (0.68 ± 0.06 g/g DM; $P \geq 0.01$), followed by *S. pulchra* (0.64 ± 0.08 g/g DM; $P \geq 0.01$) and *B. nana* (0.55 ± 0.05 g/g DM; $P > 0.01$). Dry matter

digestibility did not vary among ecoregions ($F_{2, 376} = 0.86$; $P > 0.05$) even though ecoregion was included in the final model (Appendix III).

Gross energy content was modified by variation in DM digestibility so that large gradients in digestible energy were created among forage plants over the course of the growing season. Digestible energy content varied significantly among plant groups across ordinal date ($F_{2, 191} = 7.04$; $P < 0.01$; Fig. 3) and followed patterns that were similar to those for DM digestibility (Fig. 4). Digestible energy content was highest in the forb both at the onset of the growing season and at senescence (Table 1). Deciduous shrub species had intermediate digestible energy content at the onset of the growing season and at senescence (Table 1). Graminoids contained the lowest digestible energy content at plant emergence and at senescence (Table 1). Digestible energy content varied among species of graminoids over ordinal date (Kruskal-Wallis test; $P < 0.01$): *C. bigelowii* contained higher contents of digestible energy (9.71 ± 1.53 kJ/g DM) than either *C. aquatilis* (8.62 ± 1.20 kJ/g DM) or *E. vaginatum* (8.70 ± 1.49 kJ/g DM). Digestible energy content did not vary among ecoregions ($F_{2, 191} = 2.05$; $P > 0.05$) even though ecoregion was included in the final model.

Dynamics of Fibre and Phenolic Compounds

Plant fibre (ADF), which caused the most widespread reductions in nutrient availability of forage plants to caribou, varied in a predictable manner over the course of the growing season and among plant groups. Contents of ADF varied among plant groups over ordinal date ($F_{2, 320} = 12.02$; $P < 0.01$). Graminoids had higher fibre content than either deciduous shrubs or the forb at the start of the growing season (Table 1). However, deciduous shrubs gained fibre at a faster rate than graminoids, and attained similar contents of fibre by senescence (Table 1). In contrast, the forb *Pedicularis* spp. had the lowest fibre contents at senescence (Table 1). Acid detergent fibre

content varied among ecoregions over ordinal date ($F_{2, 320} = 10.52$; $P < 0.01$), and was higher in the Arctic Foothills than either the Brooks Range or Coastal Plain (Coastal Plain: 29.60 ± 0.50 g/100g DM; Arctic Foothills: 30.54 ± 0.37 g/100g DM; and Brooks Range: 29.31 ± 0.44 g/100g DM; $F_{1, 320} \geq 8.88$; $P < 0.01$).

Phenolic compound content, unlike fibre content, was highly variable among forage plants, seasons, and years and was associated with drastic declines in nutrient availability for only a few forage plants. Phenolic compound content was typically higher in the deciduous shrubs than in the other forage plants. Phenolic compounds contents were higher in *B. nana* (13.86 ± 5.80 mg Gallic Acid Equivalents/g DM) and *S. pulchra* (43.76 ± 22.28 mg Gallic Acid Equivalents/g DM) than in graminoids (6.29 ± 0.92 mg Gallic Acid Equivalents/g DM), *Pedicularis* spp. (7.25 ± 1.45 mg Gallic Acid Equivalents/g DM), and *S. richardsonii* (6.55 ± 0.60 mg Gallic Acid Equivalents/g DM). Phenolic compound content of *B. nana* increased with ordinal date in both 2012 and 2013 (Spearman's rho = 0.77; $P < 0.01$; Fig. 6). Although phenolic compound content of *S. pulchra* varied with ordinal date, the slope varied with year (Fig. 6): phenolic compound content in *S. pulchra* was negatively correlated with ordinal date in 2011 (Spearman's rho = -0.74; $P < 0.01$) and positively correlated with ordinal date in 2012 (Spearman's rho = 0.74; $P = 0.01$). However in 2013, phenolic compound content in *S. pulchra* did not change with ordinal date ($P > 0.05$; Fig. 6).Contrasting phenological trajectories among years for *S. pulchra* will make it difficult to predict any nutritional effects caused by phenolic compounds at any given point in the growing season.

Spatiotemporal variation in $\delta^{15}N$ and $\delta^{13}C$

Plant groups differed in $\delta^{15}N$ values (range = -9.47 ‰ to +3.82 ‰; $F_{2, 97} = 140.56$; $P <$ 0.01; Appendix I; Fig. 7), because graminoids were enriched in ^{15}N (mean ± SD: 1.87 ‰ ± 1.02

‰), whereas deciduous shrubs and *Pedicularis* spp. (-2.87 ‰ ± 2.93 ‰) were depleted in ^{15}N. Values of $\delta^{15}N$ also varied among deciduous shrubs species (Kruskal-Wallis test; $P < 0.01$): *B. nana* was most depleted in ^{15}N (-7.38 ± 1.60 ‰), *S. richardsonii* was intermediate in $\delta^{15}N$ values (-4.54 ± 1.15 ‰), and *S. pulchra* was most enriched in ^{15}N (-2.87 ± 0.95 ‰). Values of $\delta^{15}N$ did not vary among ecoregions ($F_{2, 97} = 2.65$; $P > 0.05$) even though ecoregion was included in the final model (Appendix III). Season (early or late) was also not included in the final model for $\delta^{15}N$ (Appendix III).

The range of values for $\delta^{13}C$ was smaller than for $\delta^{15}N$ (5.60 ‰ vs. 13.29 ‰, respectively). Plant groups differed in $\delta^{13}C$ values ($F_{2, 96} = 23.33$; $P < 0.01$; Appendix I; Fig. 7), and there was much more variation among individual species in $\delta^{13}C$ than in $\delta^{15}N$. Graminoids were most enriched in ^{13}C (-26.57 ± 1.01 ‰), intermediate in deciduous shrubs (-27.61 ‰ ± 1.25 ‰), and most depleted in *Pedicularis* spp. (-28.27 ± 1.02 ‰). Values of $\delta^{13}C$ varied among graminoids (Kruskal-Wallis test; $P < 0.01$), as *C. aquatilis* was slightly more depleted (-27.39 ± 1.16 ‰) than *C. bigelowii* (-25.97 ± 0.56 ‰) and *E. vaginatum* (-26.18 ± 0.43 ‰). Values of $\delta^{13}C$ also varied among deciduous shrub species (Kruskal-Wallis test; $P < 0.01$): *B. nana* was the most depleted (-28.69 ± 0.59 ‰), *S. richardsonii* was intermediate (-27.94 ± 0.97 ‰), and *S. pulchra* was least depleted (-26.94 ± 1.25 ‰). Values of $\delta^{13}C$ also differed by ecoregion ($F_{2, 96} = 15.71$; $P < 0.01$) and season ($F_{1, 96} = 23.97$; $P < 0.01$): $\delta^{13}C$ values were progressively more depleted from the Brooks Range and Arctic Foothills (-26.77 ± 1.24 ‰ and -27.14 ± 1.14 ‰, respectively) to the Coastal Plain (-28.15 ± 1.21 ‰; Appendix I) and from plant emergence (-26.85 ± 1.11 ‰) to senescence (-27.29 ± 1.30 ‰).

Effect of nutrient availability on caribou $\delta^{15}N$ and $\delta^{13}C$ values

In order to determine whether sampled caribou tissues and feces may be biased in isotopic values according to diet quality, we assessed effects of nutrient availability on fractionation values between the whole plant versus the fraction of the plant that was digestible (present in animal tissues) and indigestible (present in feces). Across plant groups, fractionation of $\delta^{15}N$ between the whole plant and the indigestible fraction was negatively correlated with N digestibility ($F_{1, 77} = 10.59$; $P < 0.01$; $R^2 = 0.15$), with no fractionation when N digestibility was 52.6%. As N digestibility diverged away from this point for *B. nana* (N digestibility of 24.1%) and *S. richardsonii* (N digestibility of 44.2%), residues became significantly more enriched than the whole plant (*B. nana*: 2.12 ± 1.21‰ and *S. richardsonii*: 0.75 ± 0.44‰; both $P \leq 0.01$). This increase in diet-indigestible fractionation with decreasing N digestibility was partially caused by interference from phenolic compounds in forage plant species which contained high phenolic contents, because fractionation of $\delta^{15}N$ between the diet and the indigestible fraction was also negatively correlated with phenolic compound content for *B. nana* and *S. pulchra* ($R^2 = 0.62$ and 0.56, respectively; both $P \leq 0.01$; Fig. 8). In comparison with the diet, digested fractions (present in animal tissues) were depleted for *B. nana* (-10.48 ± 12.98 ‰; $P = 0.03$), *E. vaginatum* (-0.73 ± 1.31 ‰; $P = 0.04$), and *S. richardsonii* (-0.82 ± 0.56 ‰; $P = 0.03$; Fig. 7). Although we cannot truly say that these values represent diet-tissue or diet-feces fractionation because variation from animal endogenous substances has been eliminated, it is quite likely that these fractionation values depending on diet quality persist within whole biological systems and this factor is likely to be a significant cause of variation among fractionation values for herbivores.

Fractionation of $\delta^{13}C$ between the whole plant and the digestible and indigestible fractions was also related to forage plant quality. Across plant groups, fractionation of $\delta^{13}C$

between the diet and the indigestible fraction was negatively correlated with C digestibility ($F_{1, 75}$ = 40.14; R^2 = 0.30; P < 0.01), and no fractionation of $\delta^{13}C$ was predicted at a C digestibility of 37.4%. As forage plants diverged away from this point for *B. nana* (C digestibility of 38.0%) and *Pedicularis* spp. (C digestibility of 68.2%), significant diet-indigestible fraction was observed for these species (*B. nana*: -0.42 ± 0.28 ‰; *Pedicularis* spp.: -1.59 ± 0.46 ‰; both P < 0.01). On the other hand, in comparison with the diet, the digestible fraction was more enriched in ^{13}C for *B. nana* (0.62 ± 0.38 ‰; t_9 = -5.14; P < 0.01) and *Pedicularis* spp. (0.78 ± 0.35 ‰; t_{13} = -8.21; P < 0.01; Fig. 7). Variable $\delta^{13}C$ fractionation among animal tissues and feces according to diet quality contribute to making $\delta^{13}C$ a poor choice for isotopic dietary analyses for herbivores within arctic ecosystems.

Discussion

Digestible Nitrogen

Digestible N content is likely to be the most limiting nutritional factor for arctic herbivores because digestible N content, unlike digestible energy, only met maintenance and reproductive N requirements for a very short period of time near the beginning of the summer. Also, by the end of the growing season all forage plants failed to meet maintenance N requirements for caribou, often due to senescence (e.g., *B. nana* and *Pedicularis* spp.). In contrast, digestible energy content of deciduous shrub species and the forb *Pedicularis* spp. was sufficient to meet energy requirements for both maintenance and reproduction throughout the entire growing season (Fig. 2). Even though graminoids had relatively low digestible energy content, caribou would likely be able to increase their digestible N intake if they grazed selectively on highly-digestible inflorescences (Klein 1990) when they were available.

N digestibility was reduced by variable amounts of fibre and phenolic compounds in forage plants. Although deciduous shrub species contained the highest total N contents, the digestible N contents of willows were as low as those in graminoids, which contained more digestible N than the deciduous shrub *B. nana* (Appendix 1). In contrast, McArt et al. (2009) reported that digestible N contents were relatively high among *B. nana* compared to several other deciduous shrub species including *Salix pulchra* and *S. richardsonii*. However, McArt et al. (2009) predicted digestible N content from the activity of tannins, whereas in our study we measured N lost through digestion using purified enzymes and acid, which allowed us to account for effects of both PSMs (including tannins) and plant fibre. Differences between our observations and those of McArt et al. (2009) may also be due to differences in growing conditions between the arctic tundra and the boreal forest that may have affected the carbon balance for *B. nana* (Dormann 2003). Regardless, in our study, N digestibility was limited by both fibre (ADF) and PSMs (phenolic compounds, including tannins), because all forage plants showed decreased N digestibility with increasing ADF content; this is consistent with studies of foods consumed by domestic goats and pigs (Vencl 1992, Degen et al. 2007). Nitrogen digestibility was further suppressed by phenolic compounds in *B. nana*, which may have accounted for the extremely low digestible N content of this forage species. Low contents of digestible N in *B. nana* may explain why this forage species is seldom used by caribou (Kuropat 1984), moose (Risenhoover 1989), and muskoxen (Robus 1981) even though caribou are able to consume another shrub birch, *B. glandulosa* (Manseau et al. 1996, Thompson and Barboza 2013).

Predicting nutrient reductions in forage plants due to phenolic compounds will be difficult, because phenolic content, unlike fibre content, appears to be much more variable

among forage plants over time. Phenolic content was consistently low in graminoids but varied widely among deciduous shrub species, and even within the same species among years (Fig. 6). The large interannual variation in phenolic contents in *S. pulchra* has not been reported previously for arctic ecosystems, but large spatial differences in plant defense compounds within species are quite common for *Eucalyptus*, *Salix*, and *Betula* growing in other places (Wiggins et al. 2006, McArt et al. 2009, Bryant et al. 2014). Although deciduous shrub species are consistent in whether they produce PSMs, the content of PSMs in plant tissues can vary greatly depending on soil nutrient limitations (Bryant et al. 1987), growing conditions, and past browsing history (Stark et al. 2007).

PSM content is not the only important factor affecting nutrient availability, because PSMs are a broad class of anti-herbivory compounds that do not consistently reduce nutrient availability to herbivores (Waghorn and McNabb 2003, Iason 2007). In arctic ecosystems, the specific plant defense compounds that alter foraging behavior of herbivores have only been well-characterized for birches (Bryant et al. 1987, Stark et al. 2007). Herbivore responses, such as avoidance or acclimation, to PSMs in willows have been characterized, but the specific compounds that elicit these responses have not been characterized as well as those in birches (Reichardt 1981, Williams et al. 1992, JulkunenTiitto et al. 1996). Uncertainty in the suite of phenols present among individual deciduous shrub species and over time, as well as the mechanisms by which these phenols operate, combine to make it difficult to predict phenol-specific N reductions without actually measuring the digestible N content of the forage plant growing under the conditions in question.

Measuring Dietary Intake with Stable Isotopes of Carbon and Nitrogen

Although both C and N isotopes can be used to discriminate between monocot (graminoids) and dicot (deciduous shrubs and *Pedicularis* spp.) forage plants, several factors make $\delta^{15}N$ values a more reliable indicator of diet than $\delta^{13}C$ values for arctic herbivores. Values of $\delta^{15}N$ had a 42% greater range than values of $\delta^{13}C$ for monocot and dicot forage plants, consistent with other studies of arctic plants (Barnett 1994, Kristensen et al. 2011, Pattison and Welker 2014). Values of $\delta^{15}N$ remained constant across the season and between years, unlike those of $\delta^{13}C$, which declined over the season and from the Brooks Range to the Coastal Plain, probably due to differing levels of water stress (Chapin et al. 2011). Values for $\delta^{15}N$ varied between deciduous shrub species, probably reflecting differences in mycorrhizal associations (Nadelhoffer et al. 1996).

Although differences in growing conditions can create useful distinctions between forage groups, it is still important to consider how those signatures may change during digestion because digestible and indigestible fractions can display differing stable isotope ratios that would then be incorporated into animal tissues and feces. In particular, we found that fractionation depended upon forage plant quality because it correlated with nutrient digestibility of forage plants. Digestibilities of both C and N showed a positive relationship with fractionation, but significant fractionation between diet and indigestible residues was only observed when digestibilities diverged from 52.6 % for N and 37.4 % for C. Fractionation was only significant for *B. nana*, *S. richardonii*, and *Pedicularis* spp. as these species diverged away from the zero fractionation values. Forage plants of differing digestibility may also be represented in animal tissues at different rates and in different proportions, even if they are consumed at the same rate. For example, (Codron et al. 2011a) found that incorporation rates of ^{13}C isotopes were fastest

when animals consumed highly digestible diets, and that isotopic composition of various herbivore tissues were skewed according to the digestibility of the diet. In our study, fractionation between diet and the digested (and presumably absorbed) fraction increased when digestibilities were low. For example, fractionation of 10.5‰ for absorbed N isotopes in *B. nana* were three times greater than the traditionally accepted diet-tissue fractionation value of +3‰, and has the potential to bias estimates of consumption towards deciduous shrubs when using isotopic analyses of tissues to reconstruct diets over large scales of space or time (Gustine et al. 2012, Mann et al. 2013).

Isotopic fractionation is affected by concordant effects of fibre and phenolic compounds through a variety of mechanisms. Digestion of N is affected by physical access of enzymes to substrates, inhibition of the enzyme and by the affinity of the enzyme for the substrate, all of which can influence N fractionation (Barboza et al. 2009). Enzyme affinity for substrate proteins probably has little effect on fractionation because protease activities are high for a wide variety of dietary proteins and because the majority of plant protein is present in the form of a single photosynthetic protein, rubisco (Robbins 1993, Barboza et al. 2009). Physical access and enzyme inhibition probably account for most of the fractionation. PSMs such as tannins can limit both physical access of dietary enzymes to protein and also inhibit the dietary enzymes themselves (Robbins et al. 1987, Barry and McNabb 1999). However, (Lorenz et al. 2014) noted that differences in binding affinity between tannins and proteins depended on the characteristics of both the tannin and the protein. Fractionation of N in woody deciduous shrubs may therefore depend upon the suite of PSMs, which changes according to species, ecoregion, and season and would contribute to the uncertainty in estimates of diet from derived from isotopic analysis of feces or tissues. By correcting for differences in diet-tissue or diet-feces fractionation values

using either known phenolic content or nutrient digestibility, estimates of dietary reconstructions for herbivores will become more accurate than using a standard fractionation value.

Ability of Caribou to Cope with Low Nutrient Abundance

Caribou are able to alter their energy and N balance by selective foraging. The largest opportunity for selection exists early in the season. The forb *Pedicularis* spp. consistently had the highest digestible contents of both energy and N, which may explain why this species and other forbs are highly preferred among caribou (Russell et al. 1993). Unfortunately, this species is not abundant so herbivores must also rely on less digestible species of graminoids and deciduous shrubs. Graminoids and willows were similar in their ability to meet caribou N requirements for maintenance until late lactation in mid-August. Digestible N contents of *B. nana* were only sufficient to satisfy maintenance requirements until peak lactation (late June; Fig. 3), which is consistent with estimates of low N intake from *B. glandulosa* in captive caribou (Thompson and Barboza 2014). Deciduous shrub species contained much higher contents of digestible energy than graminoids, but the inflorescences of *E. vaginatum* and other graminoids may be higher in digestible energy than the whole plants that we sampled in our study. Selective consumption of inflorescences from *E. vaginatum* may be sufficient to meet both maintenance and reproductive requirements, but the availability of those flowers is less than that of leaves and varies widely between years depending upon growing conditions in the summer range (Shaver et al. 1986).

After selecting for type of forage plant eaten, it may also be beneficial for caribou to take advantage of foraging opportunities on a larger spatial scale. Although overall digestible N and digestible energy content in forage plants (i.e., g/g DM) did not vary among ecoregions, variation in biomass, species composition and feeding conditions among regions can produce spatial gradients in total amounts of digestible nutrients (i.e., g/m^2) and the ability of caribou to use

those gradients across the summer range. For example, the Coastal Plain is dominated largely by graminoid marshes (Gallant et al. 1995), which provide a toxin-free digestible N supply. Young graminoids high in digestible N content can be found here at a reliable time across years because the start of the growing season is much more consistent in the Coastal Plain ecoregion than further inland. If total biomass of low-toxin graminoids in the Coastal Plains ecoregion is high enough, then digestible N (g/m^2) may very well be higher in this ecoregion compared to more southerly ecoregions, which are more dominated by high-toxin deciduous shrubs. Indeed, female caribou of the Central Arctic herd migrate to the Coastal Plain to calve; this ecoregion offers several other advantages for reproductive females: predator and insect harassment levels are typically lower than in the Arctic Foothills and Brooks Range ecoregions (Young et al. 2002). Caribou and reindeer who are harassed less by insects and predators also face lower energy expenditures and can forage for an increased amount of time (Hagemoen and Reimers 2002, Colman et al. 2003, Witter et al. 2012), which may further increase summer nutrient gains (Weladji et al. 2006).

Arctic Caribou Nutrition in a Changing Climate

Caribou nutrition will be affected by changing forage plant dynamics predicted as a result of a changing climate. Projected increases in abundance of shrubs (Myers-Smith et al. 2011), in particular heavily-defended shrubs such as *B. nana* (Shaver et al. 2001, Euskirchen et al. 2009) are likely to negatively affect protein nutrition for caribou (Thompson and Barboza 2014). Changes in abundance of forbs and graminoids (Walker et al. 2006) may also affect caribou nutrition. The net effect on caribou populations of these changes may depend on the ability of individual caribou to cope with these projected changes.

Caribou may be able to cope with a changing forage plant base using several mechanisms. Coping mechanisms can take the form of salivary proteins which bind and inactivate tannins in deciduous shrubs species, however our knowledge of how these compounds are induced in ruminants is limited (Austin et al. 1989, Robbins et al. 1991, Juntheikki 1996). Some forms of toxins can be degraded by gut microbes in caribou (e.g., usnic acid; Sundset et al. 2010), however we do not know if any other forms of PSMs can be neutralized in caribou using this method. Other mechanisms of coping with PSM intake can include behavioral avoidance of PSM-rich forages (Bryant et al. 1991, McLean and Duncan 2006, Estell 2010). When feeding on deciduous shrubs, however, caribou appear unable to avoid high phenolic loads (Thompson and Barboza 2014), which can also come at a significant N cost as these toxins are removed from the body (Au et al. 2013).

The ability of individual caribou to alter their physiological or behavioral responses to fibre and phenols in forage plants may directly affect survival, growth, and reproduction, especially if vegetation in the summer ranges becomes dominated by shrubs that are defended with PSMs (Sturm et al. 2001, Myers-Smith et al. 2011). In light of a potentially changing forage base, the persistence of a particular population or herd of caribou may therefore depend on their ability to develop effective defenses against forage PSMs and the availability of non-defended forages such as graminoids and forbs. However, if heavily-defended forage plants such as *B. nana* do increase in caribou diets, our ability to detect these changes using stable isotope analyses should be high because *B. nana* contained large contents of fibre and phenolic compounds that profoundly reduced N digestibility. Low N digestibilities and high phenolic contents, such as those found in *B. nana*, resulted in a large degree of fractionation between the diet and the digested fraction that would be incorporated into the tissues of caribou. Thus, diets

of heavily-defended, low-N shrubs are likely to be overrepresented in diet estimates from animal tissues.

Conclusions

We found significant interactions between nutrients and fibre and phenolic compounds, which combined to create a complex picture of the amount of nutrients actually absorbed by animals. In particular, contents of digestible N are far below total N contents, which may reduce the ability of female caribou to obtain enough N for reproduction. Digestible energy contents, on the other hand, are generally sufficient to meet caribou requirements throughout the growing season. Caribou can gain more nutrients by feeding selectively on certain forage plants, although *B. nana* in particular yielded little or no digestible N to caribou due to digestion interference by fibre and phenolic compounds. Fibre and phenolic compounds have the ability to alter how isotopic signals are changed as they make their way from the plant into tissues or feces, and may skew isotopic estimates of diet. Isotopes of C in particular are a poor choice for a dietary marker in arctic ecosystems because any differences among plant groups are masked by seasonal, latitudinal, and changes due to fractionation. Isotopes of N, on the other hand, were relatively robust and displayed large differences among plant groups that were not as affected by fractionation changes except for one species, *B. nana*, which showed such a large fractionation between the whole plant and digested (and presumably absorbed) fraction that this may be a useful feature to detect whether caribou are beginning to utilize this food resource that could have negative implications for recruitment into caribou herds.

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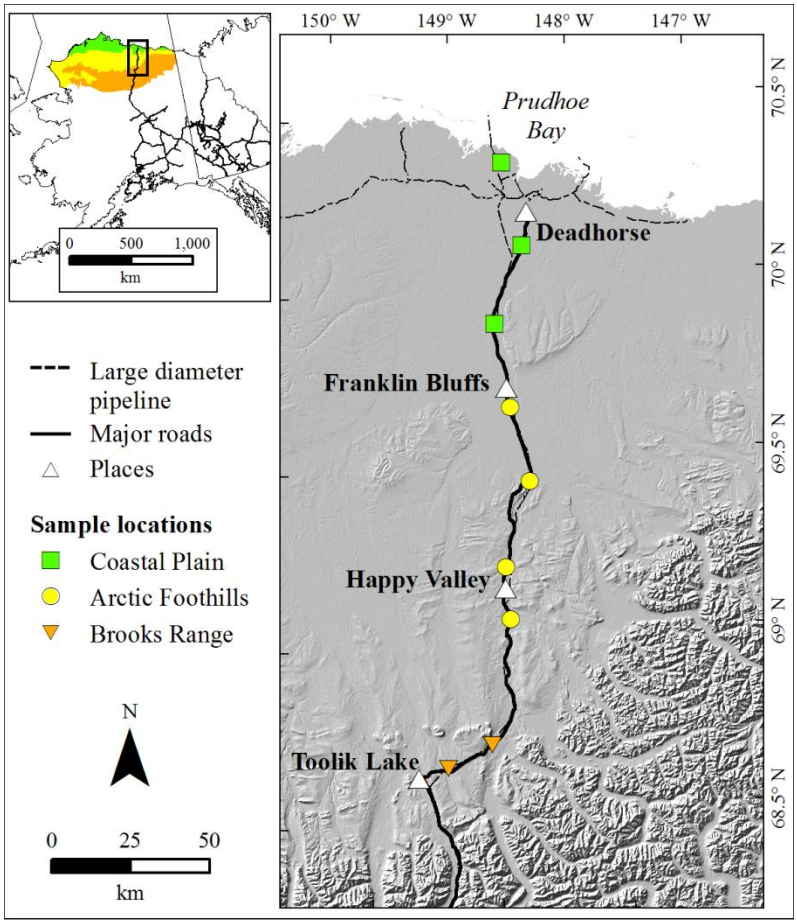

Figure 3.1. Location of study sites within the range of the Central Arctic caribou herd. Sites were located in three ecoregions (Coastal Plain, Arctic Foothills, and Brooks Range) along the Dalton Highway. The distribution of the Arctic ecoregions in Alaska is noted in the inset.

Table 3.1. Predicted mean (± SE) of nutritional variables from robust linear regressions for each plant forage group according to season. First row of each variable indicates the estimate throughout entire growing season. First indented row of each variable indicates the estimate at plant forage emergence (Ordinal date 150). Second indented row of each variable indicates the mean value at plant forage senescence for the forb (Ordinal date 240) and the graminoids and browse forage plants (Ordinal date 270). Subscripts indicate significant differences between plant forage groups. For senescent forbs, late season comparisons with other forage plant groups were made at Ordinal date 240 (not shown for deciduous shrubs and graminoids).

	Plant Group		
	Deciduous Shrub	**Forb**	**Graminoid**
Total N	$2.24 \pm 0.03_{a}$	$1.83 \pm 0.04_{b}$	$1.90 \pm 0.02_{ab}$
Early	$3.73 \pm 0.10_{a}$	$3.38 \pm 0.12_{b}$	$2.45 \pm 0.09_{ab}$
Late	$1.22 \pm 0.08_{ab}$	$1.14 \pm 0.10_{b}$	$0.68 \pm 0.04_{a}$
N Digestibility	$0.36 \pm 0.02_{b}$	$0.71 \pm 0.02_{ab}$	0.52 ± 0.01a
Early	$0.43 \pm 0.05_{ac}$	$0.89 \pm 0.03_{bc}$	$0.65 \pm 0.03_{ab}$
Late	$0.25 \pm 0.08_{a}$	$0.70 \pm 0.03_{ab}$	$0.36 \pm 0.02_{b}$
Digestible N	$0.92 \pm 9.05_{ab}$	$1.40 \pm 0.06_{b}$	$1.08 \pm 0.02_{a}$
Early	$1.87 \pm 0.16_{a}$	$3.06 \pm 0.17_{ab}$	$1.99 \pm 0.09_{b}$
Late	$0.43 \pm 0.13_{a}$	$0.82 \pm 0.11_{a}$	0.30 ± 0.03
Gross Energy	$19.57 \pm 0.11_{a}$	17.31 ± 0.13	$18.37 \pm 0.05_{a}$
Early	$19.88 \pm 0.20_{ab}$	$17.93 \pm 0.17_{b}$	$18.39 \pm 0.09_{a}$
Late	$19.27 \pm 0.18_{ac}$	$17.00 \pm 0.19_{bc}$	$18.35 \pm 0.09_{ab}$
DM Digestibility	$0.64 \pm 0.01_{ac}$	$0.84 \pm 0.01_{bc}$	$0.49 \pm 0.00_{ab}$
Early	$0.62 \pm 0.021_{ac}$	$0.94 \pm 0.03_{bc}$	$0.55 \pm 0.02_{ab}$
Late	$0.57 \pm 0.01_{ac}$	$0.85 \pm 0.02_{bc}$	$0.39 \pm 0.01_{ab}$
Digestible Energy	$12.34 \pm 0.13_{c}$	$14.86 \pm 0.34_{bc}$	$8.95 \pm 0.09_{b}$
Early	$12.54 \pm 0.27_{ac}$	$16.85 \pm 0.60_{bc}$	$10.10 \pm 0.33_{ab}$
Late	$10.34 \pm 0.41_{ac}$	$14.16 \pm 0.42_{bc}$	$7.04 \pm 0.12_{ab}$
ADF	26.76 ± 0.56	26.28 ± 0.77	31.77 ± 0.23
Early	$23.16 \pm 1.46_{a}$	$19.29 \pm 2.80_{b}$	$28.99 \pm 0.80_{ab}$
Late	$38.51 \pm 2.48_{c}$	$24.14 \pm 1.24_{bc}$	$40.30 \pm 0.74_{b}$

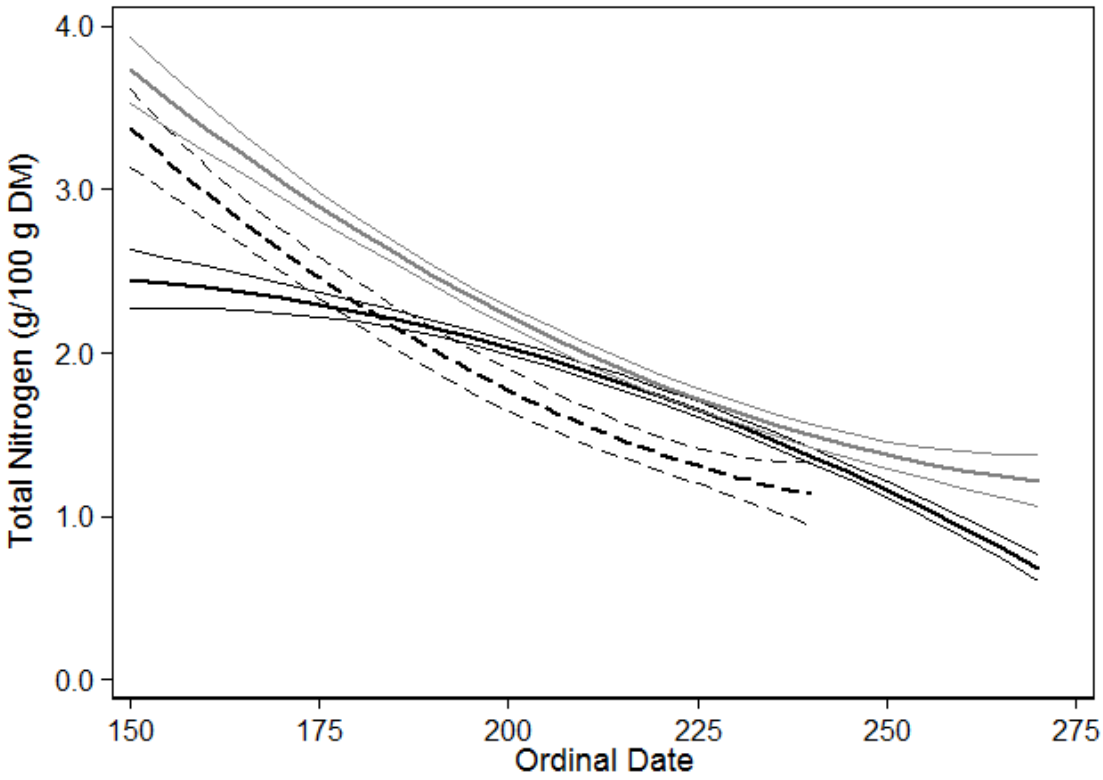

Figure 3.2. Predicted phenological progression of total N content (g/100 g DM) for graminoids (solid black lines), deciduous shrubs (solid gray lines) and *Pedicularis* spp. (broken lines) on the summer range of the Central Arctic Caribou Herd, 2011-2013. Thin lines indicate 95% confidence intervals. All relationships are in the following form: Total N = Eco PG OD Eco×PG PG×OD PG×OD^2 OD^2 where PG = plant group, Eco = ecoregion, OD = ordinal date.

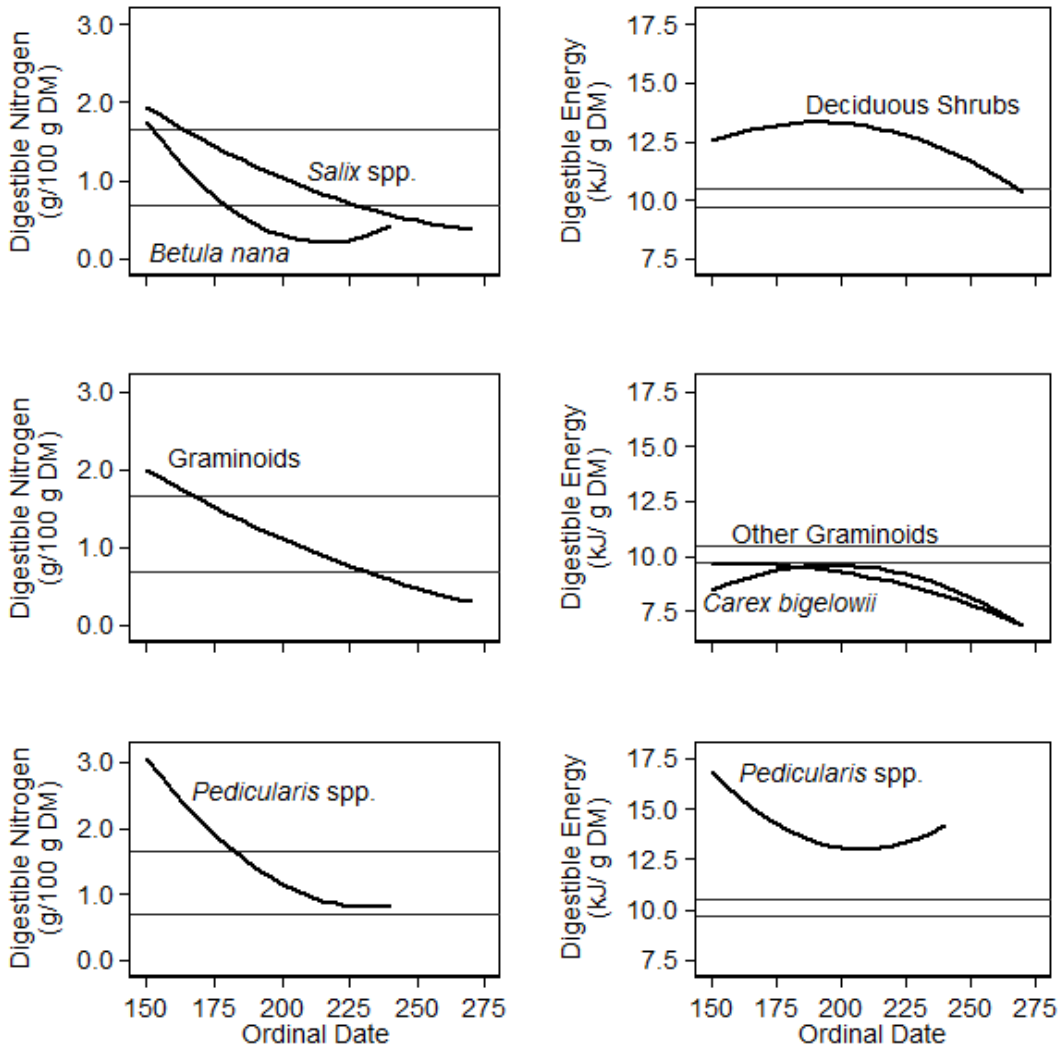

Figure 3.3. Phenological progression of digestible nitrogen (N; g/100 g DM) and digestible energy (kJ/g DM) in relation to estimated minimum nutrient requirements of caribou for deciduous shrubs (top panels; solid black lines), graminoids (middle panels; solid gray lines) and *Pedicularis* spp.(bottom panels; broken line) collected on the summer range of the Central Arctic Caribou Herd, 2011-2013. Lines are the fitted values from ordinary least-squares linear

regression. Relationships are of the following form: Digestible N = Eco PG OD Eco×PG PG×OD PG×OD^2 OD^2; Digestible energy = PG OD PG×OD PG×OD^2 OD^2; where PG = plant group, Eco = ecoregion, OD = ordinal date. *Salix* spp. contained significantly more digestible N than *Betula* (P< 0.01; top left panel). *Carex bigelowii* contained significantly more digestible energy than *C. aquatilis* and *Eriophorum vaginatum* (i.e., Other Graminoids; middle right panel) over ordinal day. Horizontal lines on each panel indicate minimum dietary contents of digestible N and digestible energy required to satisfy the requirements for maintenance (bottom line: 0.69 g digestible N/100 g DM, 9.68 kJ/g DM) and both maintenance and reproduction of female caribou (top line: 1.66 g digestible N/100 g DM, 10.49 kJ/g DM; Barboza unpublished data).

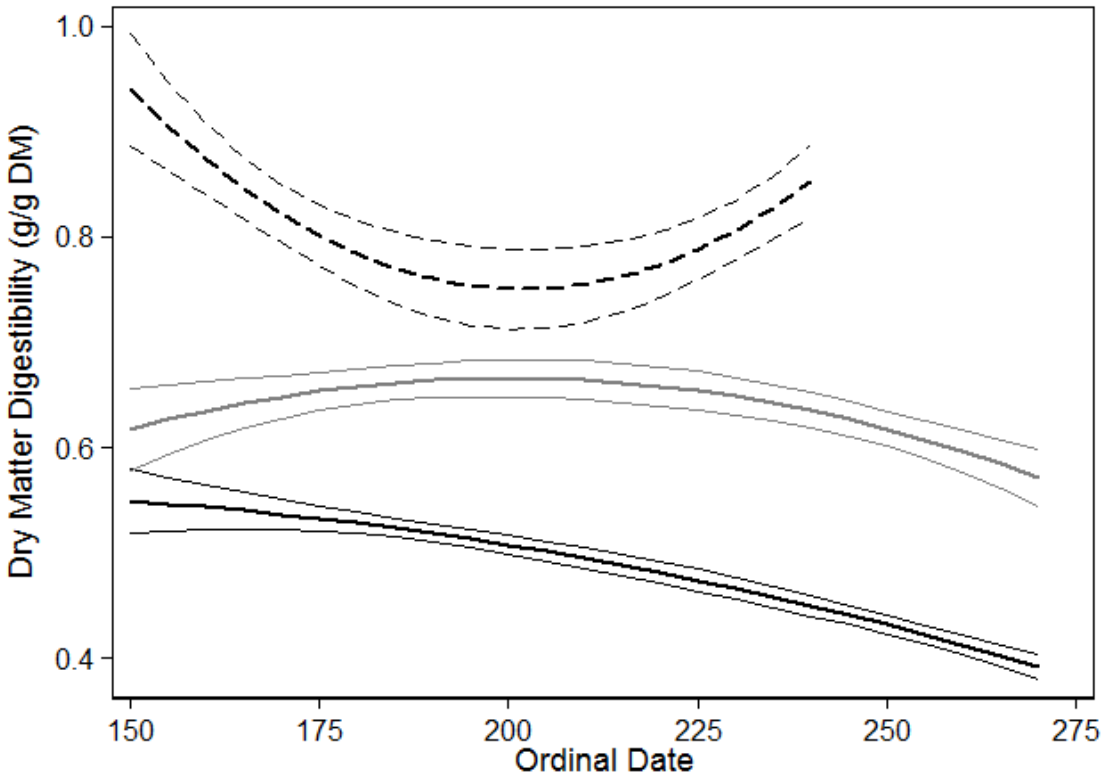

Figure 3.4. Predicted phenological progression of dry matter digestibility (g/g DM) for graminoids (solid black lines), deciduous shrubs (gray lines), and *Pedicularis* spp. (broken lines) on the summer range of the Central Arctic Caribou Herd, 2011-2013. Thin lines indicate 95% confidence intervals. Relationships are of the following form: Dry matter digestibility = Eco PG OD Eco×PG PG×OD PG×OD^2 OD^2; where PG = plant group, Eco = ecoregion, OD = ordinal date.

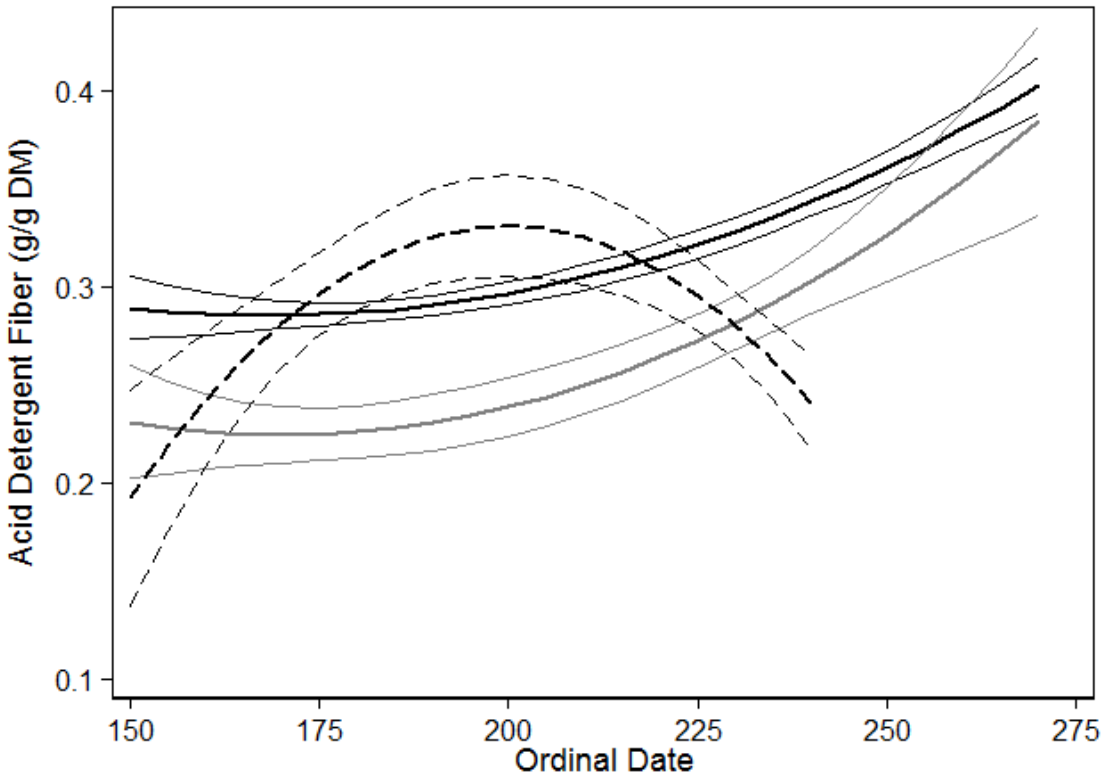

Figure 3.5. Predicted phenological progression of acid detergent fibre (ADF) content (g/g DM) for graminoids (solid black lines), deciduous shrubs (gray lines), and *Pedicularis* spp. (broken lines) on the summer ranges of the Central Arctic Caribou Herd, 2011-2013. Thin lines indicate 95% confidence intervals. Relationships are of the following form: ADF = Eco PG OD Eco×PG PG×OD PG×OD^2 OD^2; where PG = plant group, Eco = ecoregion, OD = ordinal date.

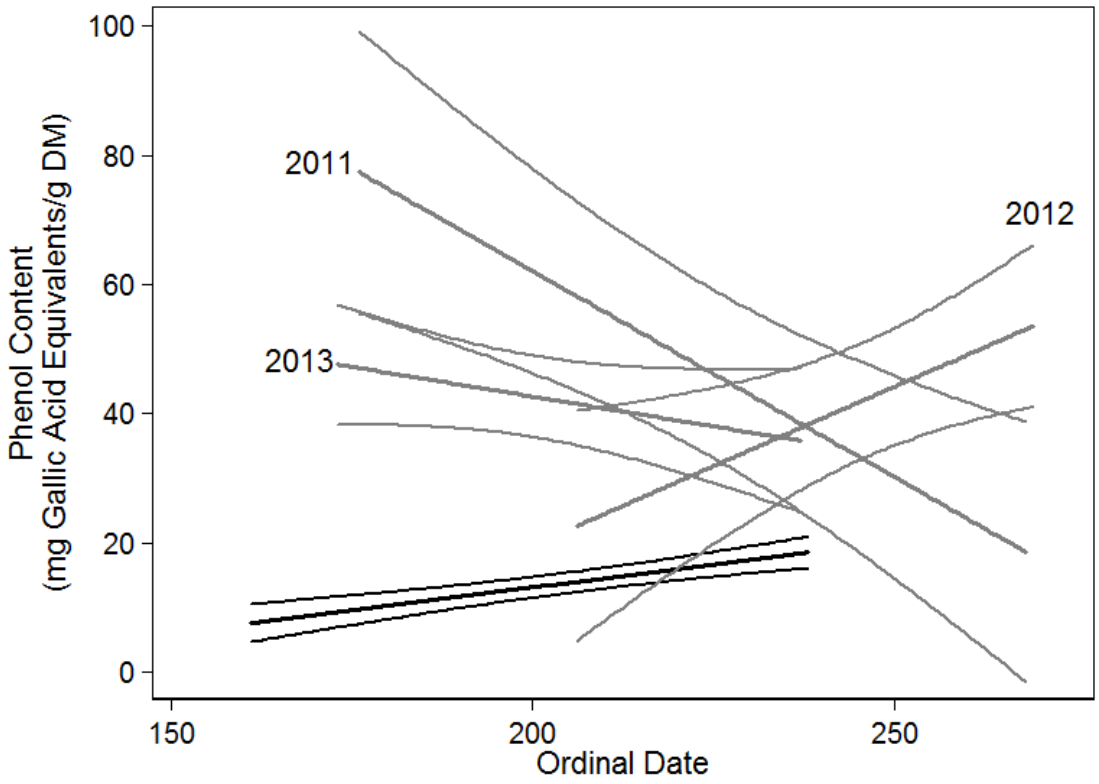

Figure 3.6. Phenological progression of phenolic content (mg Gallic Acid Equivalents/g DM) in two species of deciduous shrubs: *Betula nana* (black lines) collected during 2012 - 2013 and *Salix pulchra* (gray lines) collected during 2011 - 2013. Contents of phenolic compounds in other species were below 6.60 ± 1.06 mg Gallic Acid Equivalents/g DM. Thin lines indicate 95% confidence intervals.

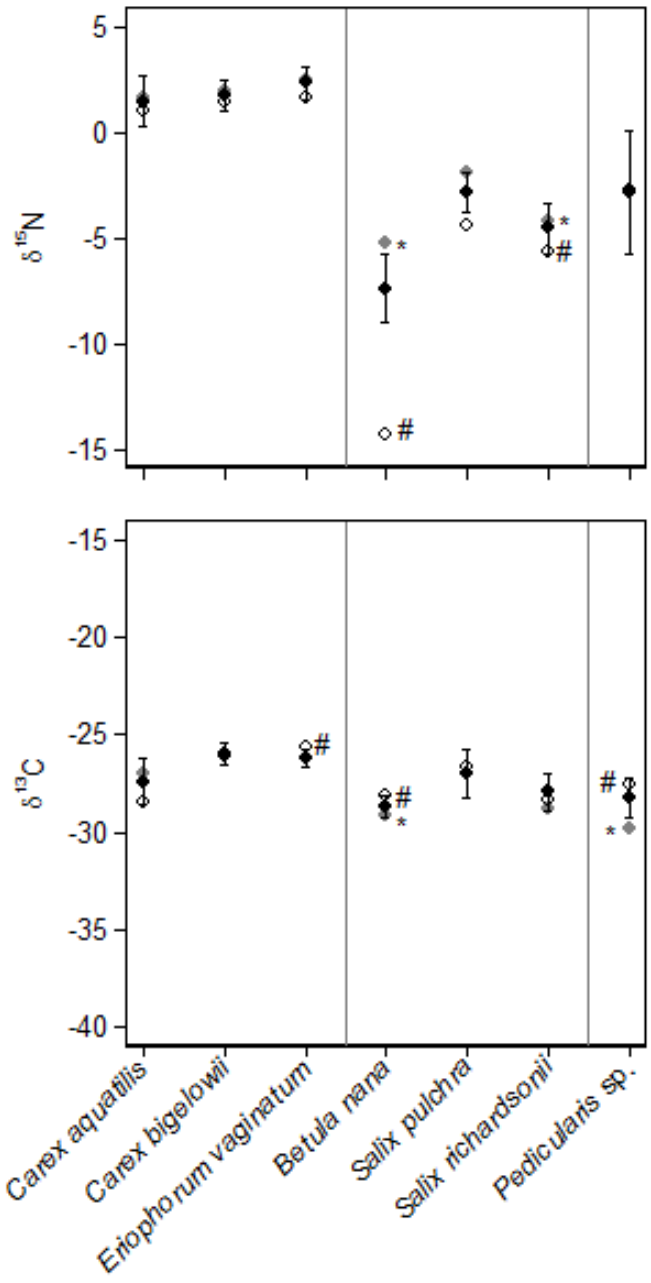

Figure 3.7. Values for $\delta^{15}N$ and $\delta^{13}C$ in whole plants (black dots), indigestible residues (gray dots), and the digested fraction (hollow dots) of caribou forages collected from the summer range of the Central Arctic Caribou herd, 2011-2012. Symbols (*) next to gray dots indicate significant

differences between indigestible residue and whole plant. Symbols (#) next to hollow dots indicate significant differences between the digested fraction and whole plant. Indigestible residues were obtained from a microbe-free digestion procedure. The values for the digested fraction were calculated from the change in mass of each isotope between whole plant and indigestible residue. Lines and whiskers represent ± 1 SD from the mean isotopic values of the whole plant.

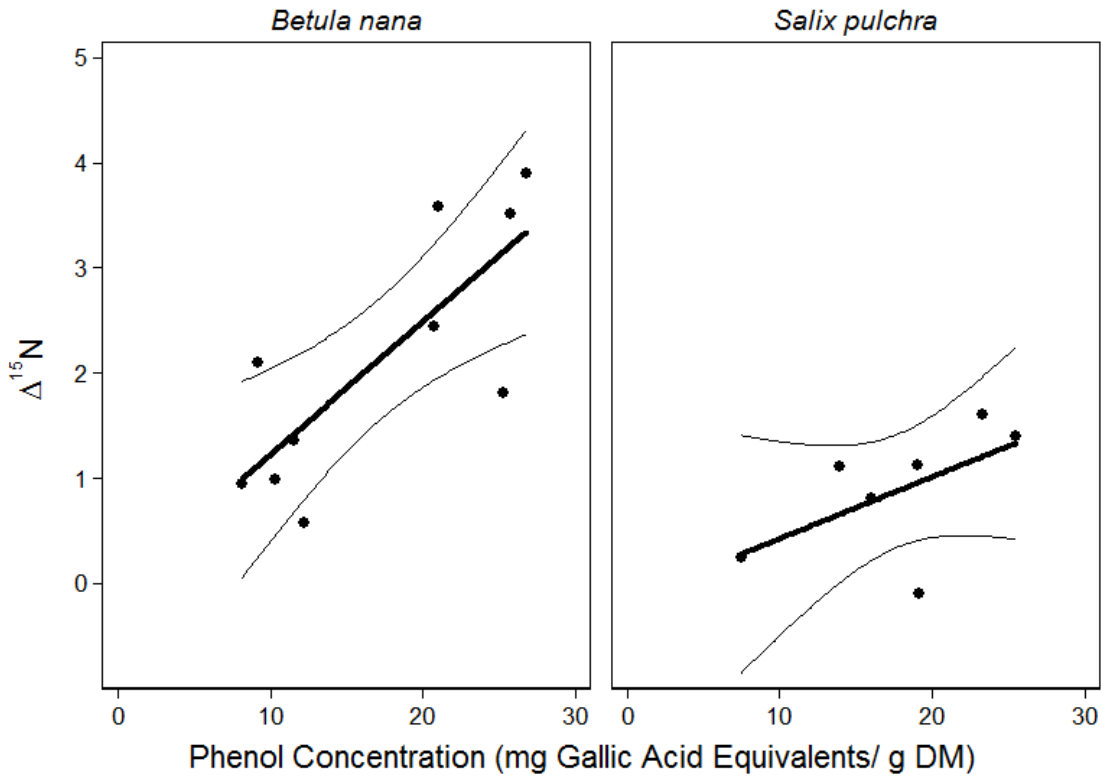

Figure 3.8. Relationship between diet-residue fractionation of $\delta^{15}N$ and phenolic content of two species of deciduous shrubs: *Betula nana* ($Y = 0.13\,x - 0.04$; $R^2 = 0.62$; $P = 0.01$) and *Salix pulchra* ($Y = 0.03\,x + 0.22$; $R^2 = 0.56$; $P < 0.01$). Although diet-residue fractionation of $\delta^{15}N$ was significant for *S. richardsonii* ($P < 0.01$) the fractionation was not correlated with phenolic content. Thin lines indicate 95% confidence intervals.

Appendix 3.1. Nutrient contents in the summer range of the Central Arctic caribou herd, 2011-2013. Dry matter digestibility was calculated using regression equations found in VanSomeren et al (in review) to predict *in sacco* DM digestibility from purified enzyme *in vitro* DM digestibility measured in our study. *Betula nana* was not found at sampling sites on the Coastal Plain.

Total Nitrogen (g/100 g DM)						
Forage Species	**Ecoregion Brooks Range**		**Arctic Foothills**		**Coastal Plain**	
	Mean ± SD	*N*	Mean ± SD	*N*	Mean ± SD	*N*
Betula nana	2.02 ± 0.50	22	1.95 ± 0.54	32		
Carex aquatilis	1.84 ± 0.67	16	1.85 ± 0.62	52	2.05 ± 0.66	52
Carex bigelowii	2.12 ± 0.75	40	1.84 ± 0.68	63	2.10 ± 0.65	22
Eriophorum vaginatum	1.82 ± 0.47	44	1.74 ± 0.43	80	1.90 ± 0.47	34
Pedicularis spp.	1.95 ± 0.78	38	1.87 ± 0.69	42	2.22 ± 1.00	30
Salix pulchra	2.12 ± 0.70	50	2.20 ± 0.74	70	2.53 ± 0.74	22
Salix richardsonii	2.00 ± 0.72	5	2.03 ± 0.70	26	2.22 ± 0.84	31

Digestible Nitrogen (g/100 g DM)						
Forage Species	**Ecoregion Brooks Range**		**Arctic Foothills**		**Coastal Plain**	
	Mean ± SD	*N*	Mean ± SD	*N*	Mean ± SD	*N*
Betula nana	0.57 ± 0.45	12	0.52 ± 0.51	18		
Carex aquatilis	0.99 ± 0.51	8	1.04 ± 0.50	27	1.01 ± 0.54	26
Carex bigelowii	1.18 ± 0.63	18	1.10 ± 0.66	30	1.20 ± 0.57	13
Eriophorum vaginatum	1.03 ± 0.56	15	0.92 ± 0.49	29	0.98 ± 0.37	17
Pedicularis spp.	1.59 ± 0.92	15	1.46 ± 0.75	17	1.62 ± 0.98	11
Salix pulchra	0.88 ± 0.59	18	1.10 ± 0.57	25	1.07 ± 0.82	9
Salix richardsonii	1.41 ± 0.04	2	0.93 ± 0.56	9	1.16 ± 0.54	14

	Dry Matter Digestibility (g/g DM)					
	Ecoregion					
Forage Species	**Brooks Range**		**Arctic Foothills**		**Coastal Plain**	
	Mean ± SD	*N*	Mean ± SD	*N*	Mean ± SD	*N*
Betula nana	0.56 ± 0.05	12	0.55 ± 0.04	18		
Carex aquatilis	0.47 ± 0.07	8	0.47 ± 0.06	27	0.47 ± 0.06	26
Carex bigelowii	0.53 ± 0.07	18	0.51 ± 0.07	30	0.52 ± 0.05	14
Eriophorum vaginatum	0.48 ± 0.07	16	0.47 ± 0.06	30	0.48 ± 0.05	17
Pedicularis spp.	0.83 ± 0.10	17	0.80 ± 0.09	20	0.82 ± 0.08	17
Salix pulchra	0.64 ± 0.07	26	0.65 ± 0.08	37	0.65 ± 0.10	13
Salix richardsonii	0.65 ± 0.06	4	0.69 ± 0.07	17	0.68 ± 0.06	24

	Digestible Energy (kJ/g DM)					
	Ecoregion					
Forage Species	**Brooks Range**		**Arctic Foothills**		**Coastal Plain**	
	Mean ± SD	*N*	Mean ± SD	*N*	Mean ± SD	*N*
Betula nana	12.13 ± 1.03	6	11.89 ± 1.12	9		
Carex aquatilis	8.44 ± 1.49	6	8.70 ± 1.11	18	8.59 ± 1.26	17
Carex bigelowii	9.82 ± 1.62	12	9.48 ± 1.68	19	10.19 ± 0.87	7
Eriophorum vaginatum	9.12 ± 1.77	9	8.49 ± 1.45	20	8.77 ± 1.36	9
Pedicularis spp.	14.89 ± 1.83	9	14.11 ± 1.45	10	14.55 ± 1.52	5
Salix pulchra	12.19 ± 1.57	14	12.02 ± 1.66	17	12.87 ± 2.15	5
Salix richardsonii	13.71	1	12.42 ± 1.03	6	12.20 ± 0.80	5

$\delta^{15}N$						
Forage Species	**Ecoregion Brooks Range**		**Arctic Foothills**		**Coastal Plain**	
	Mean ± SD	*N*	Mean ± SD	*N*	Mean ± SD	*N*
Betula nana	-5.89 ± 0.74	4	-8.37 ± 1.14	6		
Carex aquatilis	2.04 ± 0.60	2	1.91 ± 1.37	7	0.74 ± 0.84	6
Carex bigelowii	2.38 ± 0.68	4	1.32 ± 0.45	6		
Eriophorum vaginatum	2.44 ± 0.31	4	2.06 ± 0.76	8	2.73 ± 1.23	4
Pedicularis spp.	-3.62 ± 0.54	4	-3.39 ± 0.77	6	-2.63 ± 1.30	4
Salix pulchra	-2.20 ± 0.54	8	-3.39 ± 0.77	12	-2.63 ± 1.30	4
Salix richardsonii	-2.24 ± 0.82	2	-5.45 ± 0.57	6	-4.44 ± 0.54	8

$\delta^{13}C$						
Forage Species	**Ecoregion Brooks Range**		**Arctic Foothills**		**Coastal Plain**	
	Mean ± SD	*N*	Mean ± SD	*N*	Mean ± SD	*N*
Betula nana	-28.42 ± 0.59	4	-28.88 ± 0.56	6		
Carex aquatilis	-27.73 ± 0.11	2	-26.92 ± 0.68	7	-27.82 ± 1.62	6
Carex bigelowii	-25.58 ± 0.53	4	-26.22 ± 0.44	6		
Eriophorum vaginatum	-25.58 ± 0.53	4	-26.22 ± 0.44	6	-26.67 ± 0.53	4
Pedicularis spp.	-28.04 ± 1.30	4	-28.12 ± 1.10	6	-28.74 ± 0.61	4
Salix pulchra	-26.19 ± 0.70	8	-26.85 ± 0.91	12	-28.73 ± 1.37	4
Salix richardsonii	-26.36 ± 0.91	2	-27.65 ± 0.63	6	-28.56 ± 0.62	8

Appendix 3.2. Predictive relationships of digestible N (*X*; g/100 g DM) in relation to total nitrogen (*Y*; g/100 g DM), and acid detergent fiber (*Z*; g/100 g DM). Phenolic content (*P*; mg Gallic Acid Equivalents/g DM) is included in the models for *B. nana* and *S. pulchra* because these were the only species to contain phenols in significant amounts. All models are significant with $P < 0.01$.

Forage Species	**Model**	R^2
Betula nana	$X = 0.7530Y - 0.2143Z + 0.0134P - 1.0054$	0.91
Carex aquatilis	$X = 0.7131Y + 1.1497Z - 0.7011$	0.91
Carex bigelowii	$X = 0.7731Y + 0.8993Z - 0.6983$	0.94
Eriophorum vaginatum	$X = 0.6760Y - 1.3408Z + 0.1356$	0.84
Pedicularis spp.	$X = 0.8672Y - 2.5709Z + 0.4308$	0.97
Salix pulchra	$X = 0.3126Y - 2.2903Z - 0.0031P + 0.8506$	0.82
Salix richardsonii	$X = 0.6010Y - 1.2722Z - 0.7602$	0.83

Appendix 3.3. Corrected Akaike's Information Criterion (AICc) and model weights (w_iAICc) of competing models of spatial and temporal variation in nutrient and fiber contents and stable isotope values among plant groups. Robust ordinary least-squares (OLS) linear regressions were used to assess spatial and temporal variation in nutrient and fiber contents whereas variation in stable isotope values was assessed with ANOVA models. The selected model for each dependent variable with the lowest AICc score is identified in italics. In all models, PG = plant group, Eco = ecoregion, OD = ordinal date (for OLS models) and SS = subset (early or late season [for ANOVA models]).

Total Nitrogen (N = 771)				
Model	**AICc**	**w_iAICc**	**R^2**	**Number of Parameters**
Eco PG OD Eco×PG PG×OD PG×OD^2 OD^2	*843.4181*	0.5255	0.6371	*15*
Eco PG OD Eco×PG Eco×OD PG×OD PG×OD^2 OD^2	845.2164	0.2138	0.6383	17
Eco PG OD Eco×PG Eco×OD Eco×OD^2 PG×OD PG×OD^2 OD^2	845.7415	0.1654	0.6400	19
Eco PG OD PG×OD PG×OD^2 OD^2	846.8348	0.0952	0.6316	11
Eco PG OD Eco×PG PG×OD OD^2	888.8781	0.0000	0.6130	13

Nitrogen Digestibility (*N* = 336)				
Model	**AICc**	**w_iAICc**	**R^2**	**Number of Parameters**
PG OD PG×OD PG×OD^2 OD^2	-493.4420	0.4248	0.5685	9
Eco PG OD PG×OD PG×OD^2 OD^2	-492.3533	0.2465	0.5726	11
Eco PG OD Eco×PG PG×OD PG×OD^2 OD^2	-492.0463	0.2114	0.5831	15
PG OD PG×OD OD^2	-489.6168	0.0627	0.5581	7
Eco PG OD Eco×PG PG×OD OD^2	-487.8056	0.0254	0.5723	13
Eco PG OD Eco×PG Eco×OD PG×OD PG×OD^2 OD^2	-487.6642	0.0236	0.5832	17
Eco PG OD Eco×PG Eco×OD Eco×OD^2 PG×OD PG×OD^2 OD^2	-484.7411	0.0055	0.5851	19

Digestible Nitrogen (*N* = 333)				
Model	**AICc**	**w_iAICc**	**R^2**	**Number of Parameters**
Eco PG OD Eco×PG PG×OD PG×OD^2 OD^2	298.7732	0.6314	0.6801	15
Eco PG OD PG×OD PG×OD^2 OD^2	301.2309	0.1848	0.6692	11
Eco PG OD Eco×PG Eco×OD PG×OD PG×OD^2 OD^2	302.3841	0.1038	0.6809	17
Eco PG OD Eco×PG Eco×OD Eco×OD^2 PG×OD PG×OD^2 OD^2	303.6250	0.0558	0.6840	19
Eco PG OD Eco×PG PG×OD OD^2	305.3008	0.0241	0.6695	13

Gross Energy (N = 216)				
Model	**AICc**	**w_iAICc**	**R^2**	**Number of Parameters**
Eco PG OD Eco×PG PG×OD	469.7388	0.6059	0.5966	12
Eco PG OD Eco×PG PG×OD OD^2	471.9835	0.1972	0.5966	13
Eco PG OD Eco×PG	472.3195	0.1667	0.5832	10
Eco PG OD Eco×PG PG×OD PG×OD^2 OD^2	476.1662	0.0244	0.5974	15
Eco PG OD Eco×PG Eco×OD PG×OD PG×OD^2 OD^2	479.8002	0.0040	0.5994	17
Eco PG OD Eco×PG Eco×OD Eco×OD^2 PG×OD PG×OD^2 OD^2	481.3987	0.0018	0.6052	19
Eco PG OD PG×OD PG×OD^2 OD^2	498.6747	0.0000	0.5339	11

Dry Matter Digestibility (N = 391)				
Model	**AICc**	**w_iAICc**	**R^2**	**Number of Parameters**
Eco PG OD Eco×PG PG×OD PG×OD^2 OD^2	-1021.5794	0.6181	0.7870	15
Eco PG OD PG×OD PG×OD^2 OD^2	-1019.6405	0.2344	0.7812	11
Eco PG OD Eco×PG Eco×OD PG×OD PG×OD^2 OD^2	-1017.7063	0.0891	0.7873	17
Eco PG OD Eco×PG Eco×OD Eco×OD^2 PG×OD PG×OD^2 OD^2	-1016.8585	0.0583	0.7892	19
Eco PG OD Eco×PG PG×OD OD^2	-985.4940	0.0000	0.7633	13

Digestible Energy (*N* = 202)				
Model	**AICc**	**w_iAICc**	**R^2**	**Number of Parameters**
PG OD PG×OD PG×OD^2 OD^2	592.7862	0.4937	0.8336	9
Eco PG OD PG×OD PG×OD^2 OD^2	592.8050	0.4891	0.8372	11
Eco PG OD Eco×PG PG×OD PG×OD^2 OD^2	600.1110	0.0127	0.8387	15
Eco PG OD Eco×PG Eco×OD PG×OD PG×OD^2 OD^2	604.2566	0.0016	0.8392	17
PG OD PG×OD OD^2	604.9217	0.0011	0.8194	7
Eco PG OD PG×OD OD^2	604.9659	0.0011	0.8232	9
Eco PG OD Eco×PG Eco×OD Eco×OD^2 PG×OD PG×OD^2 OD^2	606.1740	0.0006	0.8415	19

Acid Detergent Fiber (*N* = 335)				
Model	**AICc**	**w_iAICc**	**R^2**	**Number of Parameters**
Eco PG OD Eco×PG PG×OD PG×OD^2 OD^2	-1113.1007	0.6466	0.5437	15
Eco PG OD PG×OD PG×OD^2 OD^2	-1110.1904	0.1509	0.5276	11
Eco PG OD Eco×PG Eco×OD PG×OD PG×OD^2 OD^2	-1109.8693	0.1285	0.5453	17
Eco PG OD Eco×PG Eco×OD Eco×OD^2 PG×OD PG×OD^2 OD^2	-1108.7658	0.0740	0.5499	19
Eco PG OD Eco×PG PG×OD OD^2	-1070.8440	0.0000	0.4756	13

δ^{15}N (*N* = 102)				
Model	**AIC**	**w_iAICc**	**R^2_{Adj}**	**Number of Parameters**
Eco PG	-220.5706	0.5490	0.7344	5
Eco PG Eco×PG	-219.1313	0.2892	0.7436	9
Eco PG SS PG×SS	-216.5573	0.0799	0.7337	8
Eco PG SS Eco×PG PG×SS	-214.6976	0.0315	0.7431	12
Eco PG SS Eco×PG Eco×SS PG×SS	-211.1833	0.0054	0.7418	14

δ^{13}C (*N* =102)				
Model	**AIC**	**w_iAICc**	**R^2_{Adj}**	Number of Parameters
Eco PG SS	-152.0998	0.5434	0.4931	6
Eco PG SS PG×SS	-151.6954	0.4439	0.5035	8
Eco PG SS Eco×PG PG×SS	-144.4278	0.0117	0.4949	12
Eco PG SS Eco×PG Eco×SS PG×SS	-139.4887	0.0010	0.4853	14

CHAPTER 4: CONCLUSION

Introduction

Consistent and accurate measures of range quality are essential for research on the relationship between animals and their foods and for effective management of wildlife. This is especially important in arctic ecosystems, where populations of large herbivores such as caribou, reindeer, and muskoxen are limited primarily by the quantity and quality of forages (Blix 2005). My goal was to improve our ability to monitor range quality for arctic ruminants by accomplishing the following objectives for the Central Arctic Caribou Herd: 1) develop and validate a more useful method to measure forage quality for caribou and other arctic herbivores, 2) assess how nutrient and anti-nutrient concentrations affect range quality for the Central Arctic caribou herd, and 3) assess sources of variation in stable isotope markers which influence their use for inferring diet of arctic herbivores. Specifically, I sought to answer the following questions:

1. How do fiber and phenolics limit N availability to caribou?
2. Is digestible N sufficient for maintenance and reproduction in Central Arctic caribou on their summer range?
3. What sources of variation could affect the use of stable isotopes to estimate diet in caribou?

Development of a New *In vitro* Digestibility Assay

In **Chapter 2**, I developed an *in vitro* method for measuring digestibility by using purified enzymes based on the procedures of Choo et al. (1981), DeGabriel et al. (2008), and Tilley and Terry (1963), and validated that method against animal-based measures of *in sacco*

and *in vivo* digestibility using adult non-reproductive reindeer. The *in vitro* method yielded dry matter (DM) digestibility values that were closer to animal-based DM digestibility measures when we used a mixture of fibrolytic enzymes (Viscozyme), rather than a single enzyme for cellulose digestion. In comparison with measures of DM digestibility in the animal, *in vitro* DM digestibility values were more accurate and more precise when samples were further digested in a solution of acid and pepsin.

The *in vitro* method offered several advantages over traditional measures of DM digestibility made within animals. *In vitro* DM digestibility measures were more consistent than those obtained from the animal-based methods, which varied over the course of the season. It is especially important for long-term range monitoring programs to give consistent measures of forage quality. The *in vitro* method uses commercially-available enzymes, which facilitates comparisons of forage quality across different ranges without the need for digestion within a single group of animals at a single point in time. The *in vitro* technique is cheaper because there is no need to maintain captive animals for measures of overall digestibility or surgically-altered animals for measures of ruminal digestibility. Additionally, it was possible to measure the digestibility of individual elements such as C and N because indigestible residues from the *in vitro* method were not contaminated with microbial and endogenous components as are indigestible residues from animal-based methods. Thus, the *in vitro* digestibility method can be used to measure the likely ranges of intakes of specific nutrients by caribou depending upon the phenological stage of forage species and the composition of the diet.

The *in vitro* digestibility method is limited in the types of forages that can be sufficiently measured. Valid measures of *in vitro* DM digestibility were obtained for all forages except for lichens; an unfortunate circumstance considering that the winter diet of caribou can be composed

largely of lichens (Boertje 1984, Russell et al. 1993, Ophof et al. 2013). We believe that this may be due to the complexity of carbohydrate polymers in the fibers of lichens (Perlin and Suzuki 1962, Svihus and Holand 2000) and the lack of specific enzymes for this substrate with the *in vitro* method. These unique fiber substrates may also pose challenges to animals, because lichen digestion seems to occur at maximal rates only after a period of acclimatization (Olsen and Mathiesen 1998, Storeheier et al. 2002), which would be consistent with induction of the appropriate enzyme activities in the ruminal community of microbes. Even within our own study animals, which were not currently being fed lichens, *in sacco* DM digestibility of lichens were low (*Cladina* sp.: 0.16 ± 0.01 g/g; *Flavocetraria* sp. 0.56 ± 0.08 g/g) compared to other studies (e.g. 0.37 ± 0.13 g/g for *Cladina rangiferina*; Thomas et al. 1984). Nevertheless, the *in vitro* method is still a valuable approach to measuring forage quality for non-lichen forages, which may be more important than lichens for supplying protein and minerals to the animal (Klein 1990), especially during the growing season when animals are gaining body mass and critical nutrient reserves for reproduction and survival (Parker et al. 2009). This method therefore allows measurement of available nutrients in forage plants during the phase of the annual cycle when caribou are in positive nutrient balance and is valuable technique for assessing summer range quality and the ability of caribou populations to persist within their present or putative ranges.

Assessment of range quality for the Central Arctic caribou herd

In **Chapter 3**, I used the *in vitro* digestibility method developed in **Chapter 2** to assess the effects of variation in nutrients and anti-nutrients on the digestibility of forages consumed by caribou. Nutrient digestibility was limited by two specific classes of anti-nutrients: fiber (ADF) and plant secondary metabolites (PSMs), which reduced nutrient digestibility in different ways according to season, species, and the type of nutrient involved. Increases in fiber content were

consistently associated with declines in digestibility of nitrogen (N) and energy in all forage species. PSMs, on the other hand, were only present in two browse species (*Betula nana* and *Salix pulchra*), and were only associated with declines in N digestibility for *B. nana*. Moreover, fiber content increased as forages progressed from vegetative to senescent phases over the season, whereas PSM content in browse species varied widely with plant phenology. In *S. pulchra*, PSM content remained constant, increased, and decreased over the growing season depending on the year. PSM content in forages may change with location due to soil nutrient availability (Bryant 1987, Dormann 2003). Although the history of mammalian or insect herbivory (Scogings et al. 2011, Ruuhola et al. 2013) can also affect PSM content of forages, apparent herbivory of plants in all three years of sample collection was low from large herbivores.

Forage species differed in their ability to meet the nutrient requirements of caribou. The highest quality forage was the forb *Pedicularis* spp., which contained enough digestible N and energy to meet requirements for reproduction (estimated at 1.66 g N/100 g DM and 10.49 kJ/g DM at the end of summer; Barboza unpublished data). Although browse species contained the highest concentrations of total N, much of it was unavailable so that willows were similar to graminoids in concentrations of digestible N. The lowest concentrations of digestible N were found in *B. nana*, which failed to meet maintenance N requirements (0.69 g N/100 g DM; Barboza unpublished data) by mid-season. Despite being low in digestible N concentration, browse species contained sufficient concentrations of digestible energy to meet reproductive requirements throughout the growing season. In contrast, graminoids were generally insufficient to meet maintenance energy demands (9.68 kJ/g DM; Barboza unpublished data) by mid-season. It is likely, however, that caribou can meet energy demands for maintenance by feeding

selectively on highly-digestible flower heads of *Eriophorum vaginatum* (Klein 1990, Johnstone et al. 2002) that are preferred by females on the calving grounds (Kuropat 1984).

The observed migration patterns of Central Arctic caribou through their summer range may take advantage of spatial discrepancies in nutrient availability despite the fact that concentrations of digestible N and energy did not change across ecoregions. Female caribou, the majority of whom are normally pregnant (Cameron et al. 2002, Lenart 2011), generally remain in the Coastal Plain ecoregion after giving birth until July before slowly migrating south (Jakimchuk et al. 1987). Compared to the Brooks Range and Foothills ecoregions, the Coastal Plain contains greater proportions of undefended graminoid and browse species (*S. richardsonii*) (Gallant et al. 1995), which may provide a greater digestible N intake for reproductive females, especially if biomass levels of these species are higher in the Coastal Plain. Low predator concentrations and insect harassment in the Coastal Plain may also allow for increased feeding opportunities, which would further increase digestible N intake. Female caribou are generally located further north than bulls throughout the growing season (Jakimchuk et al. 1987), an observation consistent with the theory that reproductive animals are N-limited and thus need to forage in areas where they are able to maximize N intake, given the fact that N requirements for reproduction in caribou are much greater than N requirements for maintenance (e.g., 110 - 130% greater; Barboza and Parker 2008). In contrast with reproductive females, bulls may instead be energy-limited and benefit more from higher levels of browse biomass in the more southerly ecoregions within our study area because digestible energy concentrations in browse species were sufficient to meet maintenance energy requirements throughout the growing season. Furthermore, large-bodied bulls may be better able to process larger quantities of biomass found in the southern ecoregions compared to smaller-bodied females (Barboza and Bowyer 2000).

Differing nutritional requirements between the sexes has also been postulated as a possible reason for sexual segregation in Svalbard reindeer and other ungulates (e.g., Loe et al. 2006).

Factors affecting diet reconstructions of herbivores using stable isotope techniques

In **Chapter 3**, I examined the extent to which stable isotopes of C and N varied among forage species, ecoregions, and time in the summer range of the Central Arctic caribou herd in order to determine which isotope(s) would be sufficient to track diet. Although values of both isotopes differed between monocot (graminoid) and dicot (browse and forb) species, ^{13}C was a poor choice as a dietary marker. The range of values of ^{13}C was 58% smaller than that of ^{15}N. Furthermore, the small differences between plant groups in values of ^{13}C were obscured by variation among ecoregions, individual species within plant groups, and across the season, which was as large as the differences among plant groups. Values of ^{15}N, however, were constant over the growing season and did not change over ecoregion.

Digestive processes also have the potential to introduce variation into dietary reconstructions. I digested forage samples using the *in vitro* digestibility assay developed in **Chapter 2** and analyzed residues to determine how digestive processes affect fractionation between the diet, indigestible residues (i.e., "feces" free of contaminating microbial and endogenous materials), and the digested component. Digestibility of both C and N was correlated with fractionation between the diet and the indigestible residue, however zero fractionation was observed at N digestibilities of 52.6% and C digestibilities of 36.6%. As digestibilities of N and C diverged away from these points, significant fractionation between the diet and the indigestible residue was observed for *B. nana*, *Pedicularis* spp., and *S. richardsonii*. Fractionation of ^{15}N between the diet and the digested fraction was also negatively correlated with phenolic content in

B. nana and *S. pulchra*. However, significant fractionation between the diet and the digested fraction was only observed for *B. nana, Pedicularis* spp., and *S. richardsonii*.

Although fractionation between the diet and the digested fraction only represents the first step in a long series of physiological processes resulting in nutrient deposition within animal tissues, there is still evidence to believe that the observed dietary fractionations persist within tissues. For example, Codron et al. (2011) noted a difference in ^{13}C incorporation rates into tissues as well as discrepancies between actual dietary composition and predicted dietary composition using ^{13}C for forages differing in digestibility. It is thus likely that the ^{13}C fractionation values we measured in *B. nana* and *Pedicularis* spp. would persist among tissues and feces of herbivores, and that diet estimates obtained using this isotope would be biased towards these forages.

It is possible that these digestive-induced differences in fractionation do not occur or are not as pronounced for ^{15}N, however, the fluxes of N are not as large as those for C. Rather, the fractionation values we observed for ^{15}N may be wiped out by the large amount of endogenous N recycling within tissues of caribou as a part of their physiological mechanism for protein conservation (Barboza and Parker 2006, Barboza and Parker 2008). Browse species, particularly *B. nana*, displayed the greatest potential for fractionation and any digestive fractionation effects that persist within animal tissues will likely only be observed for this forage type, if at all. Furthermore, if any digestive effects on fractionation are observed within tissues, this would likely depend on the nutrient digestibility and phenolic content of the particular browse species eaten.

Arctic Ungulate Nutrition in a Changing Environment

The ability of caribou to maximize nutrient intake by foraging selectively among species may be altered by a changing climate. Increasing amounts of shrubs (Epstein et al. 2000, Sturm et al. 2001, Euskirchen et al. 2009) may alter protein intake of caribou, however this is dependent on several factors. If a sufficient amount of non-shrub forages remain on summer ranges and caribou can selectively feed on these chemically undefended forages, then there may be no significant impact. Protein intake on a shrub diet is also dependent on the species of shrub involved (Chapter 2, McArt et al. 2009, Thompson and Barboza 2014): *B. nana* supplied the least amount of protein in any forage type to caribou, whereas the two willow species would have provided caribou with similar amounts of protein as graminoids. Willow species low in PSM content and high in total N content such as *Salix alaxensis* are relatively common along riparian corridors (Schickhoff et al. 2002). Although we did not measure digestible N concentrations in these browse types growing within our study area, other studies have shown that *S. alaxensis* does contain much higher concentrations of digestible nitrogen compared to more heavily-defended forages such as *B. nana* and *B. glandulosa* (McArt et al. 2009, Thompson and Barboza 2014). These communities may represent an important source of N for caribou and other herbivores. If caribou must consume greater amounts of heavily-defended forages, however, they may be able to limit N losses by expressing proline-rich salivary proteins that neutralize tannins (Shimada 2006, Estell 2010). Tannin-binding salivary proteins have been found in moose (Juntheikki 1996), mule deer, and sheep (Robbins et al. 1991), however their occurrence in caribou and other arctic herbivores and the length of time required for their induction is unknown. It is also plausible that caribou in particular may degrade phenolic compounds within their rumens using microbes as they have been demonstrated to do with usnic

acid in lichens (Sundset et al. 2010), but again we do not know the extent to which this process occurs, if at all, in caribou.

In contrast with protein intake, energy intake is likely to increase with an increasing abundance of shrubs because all three browse species we measured were sufficient to meet reproductive and maintenance demands of energy throughout the entire growing season. However, if PSMs in browses are present in sufficient quantities in the form of feeding deterrents or cause excess energy to be excreted in the urine during the process of detoxification (Barboza et al. 2009), energy intake may actually decrease. It is unknown whether or not the PSMs found in arctic browse species cause this feeding-deterrent or energy-excretion effect, however caribou may be unable to avoid intake of these PSMs if they are present (Thompson and Barboza 2014) and thus herbivores may need to rely on consumption of other non-defended forages and/or other nutrient-loss mitigation techniques in order to maintain energy intakes in the presence of these specific types of PSMs (McLean and Duncan 2006, Estell 2010, Dai et al. 2014).

Arctic Ungulate Nutrition in Response to Anthropogenic Disturbance

Migratory herbivores such as caribou may be able to use large spatial areas to maximize nutrient intake if total amounts of digestible nutrients vary over the summer range or if foraging conditions in different areas allow for increased foraging opportunities. However, their ability to do so may be reduced by barriers created by anthropogenic disturbances. Reindeer and caribou have shown avoidance of anthropogenic disturbances such as roads (Cameron et al. 2002, Beauchesne et al. 2013), forest clear-cuts (Chubbs et al. 1993), hydroelectric dams (Mahoney and Schaefer 2002), mines (Weir et al. 2007), tourist resorts (Nellemann et al. 2000), and even power lines (Tyler et al. 2014). These disturbances can have large effects on herbivore populations beyond just nutritional limitation when females are displaced from preferred feeding

grounds, particularly when displacements occur to areas where predators are more abundant (Vors et al. 2007, Leblond et al. 2013).

By contrast, caribou and reindeer populations inhabiting arctic areas with lower predator concentrations seem to fare better than their southern counterparts, especially if they are able to tolerate development and/or find suitable alternative feeding grounds. For example, caribou in the Central Arctic herd avoided oilfield infrastructure during the years immediately after its construction in the late 1970s when the herd shifted the majority of its calving and post-calving distribution to development-free areas that still remained in the Coastal Plain ecoregion (Cameron et al. 2002). Despite the initial displacement away from developed areas, the Central Arctic herd grew through the subsequent years (e.g., 5000 animals in 1978 to 70000 animals in 2010; Lenart 2011). It is unlikely that this increase would have been as large or even occurred at all if the Central Arctic herd caribou did not have access to an suitable alternative calving areas because females in the developed areas produced fewer calves than females in the undeveloped areas during this time period (Cameron et al. 2002). Additionally, caribou in the Central Arctic herd appeared to develop a tolerance toward oilfield infrastructure after a period of time because they began using areas within the oilfield complex again nearly twenty years after its construction (Cronin et al. 2000). Caribou and reindeer that are more exposed to humans and their associated infrastructures may show a greater ability to habituate to developments (Skarin and Ahman 2014). If this is also the case for caribou, the effects of novel anthropogenic challenges on other arctic caribou herds will likely depend on the extent to which development occurs throughout the remaining part of the summer range and/or access to sufficient alternative foraging areas during the period of acclimatization (Taillon et al. 2012).

Future of Arctic Herbivore Populations

Arctic herbivores currently face many challenges which are only expected to increase in the future. Increases in anthropogenic activities (Wilson et al. 2013, Skarin and Ahman 2014), ecosystem shifts (Price et al. 2013), increased frequency of natural disturbances such as wildfires (Joly 2011, Gustine et al. 2014) and winter range icing events (Tyler 2010) as well as increased parasite abundance (Laaksonen et al. 2010) caused by climate change all pose challenges to arctic herbivore populations. If populations of arctic herbivores are to persist within their existing environments, they will need to continue to use their current physiological and behavioral techniques for maximizing nutrient intake. Herbivores may need to cope with novel nutritional challenges, such as an increasing amount of PSMs in forages which may limit nitrogen availability. We can use isotopic methods to identify types of forages that animals are eating, however we cannot identify the amount of PSMs animals are exposed to with these methods. More research is needed concerning the specific types of PSMs in herbivore forages, causative mechanisms behind the expressions of these PSMs, the effects of PSMs on herbivores, and the mechanisms by which herbivores can deal with increasing amounts of these toxins.

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